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AND
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SILVER-LEAF DISEASE.—VI.

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AND

G. H. BRENCHLEY, M.A.

I.—INTRODUCTION.

Since the publication of " Silver-leaf Disease, V " by Brooks and Moore (2) further investigations on this disease have been carried out, especially as regards its incidence in plum stocks and young nursery trees, the results of which are now presented. We are again indebted to the Development Commission and the Ministry of Agriculture and Fisheries for continuing to provide facilities for this work at Cambridge and Melbourn. Certain investigations have been carried out at the East Malling Research Station and the John Innes Horticultural Institution by kind permission of Mr. R. G. Hatton and Sir Daniel Hall respectively. In the provision of plum stocks and nursery trees we have been greatly assisted by Inspectors of the Ministry of Agriculture and Fisheries, especially by Messrs. Hoare, Turnbull and Spickernell.

II. WOUND PROTECTIVES.

In " Silver-leaf Disease," IV (1) and V (2) accounts were given of investigations on the subject of wound protection in fruit trees. Numerous protectives were experimented with, but the majority proved incapable of preventing infection by *Stereum purpureum* ; in addition, some had to be discarded because of their toxic action on the tissues. In " Silver-leaf Disease, V " the following protectives were recommended : Anti-fouling Paint, Red Oxide Paint, White Lead Paint and Soft Grafting Wax. Anti-fouling Paint, as stated in " Silver-leaf Disease, V " is an excellent protective but is unfortunately toxic to the bark of young trees. For large wounds on old trees, however, it can safely be recommended. The Red Oxide and White Lead Paints recommended were prepared according to formulæ which resulted in the paints having a more suitable consistency for application to tree wounds than is the case with these paints bought ready-made.

Since the publication of " Silver-leaf Disease, V " further experiments with Red Oxide and White Lead Paints and with Soft Grafting Wax have been carried out. In addition, various tar-distillate products and a paint prepared from Bordeaux Mixture powder have been tested. There has been no change in the method of experimentation ; pruning wounds on Victoria and Czar plum branches, two to three years old, have been protected immediately after cutting, and a spore-suspension of *Stereum purpureum* applied to the surface of the protective within a few days.

The protectives described below were given an extended trial during every month of the year, while two of them, home-made White Lead Paint and Soft Grafting Wax, were tested continuously from 1925 to 1929.

Creosote. This substance, obtained from a Gas Company, is the fractional distillate of gas-tar which comes over between 180° and 250° C. As a wound protective it has the disadvantage that it has to be shaken up before application. Even after this operation it is very liquid and gives no mechanical protection whatever. It proved to be quite useless as a protective against infection by *Stereum purpureum.*

Refined Tar. This was obtained from the same source as the Creosote. It is the fractional distillate of gas-tar which comes over at temperatures above 200° C. The tar is thick and gives a good surface for some time after application. It never hardens, however, and is eventually all sucked into the vessels of the wood. Its record as a protective against *Stereum purpureum* is poor, although better than that of Creosote.

Commercial Tar-distillates, A and B. These tar-distillate products are widely used in dilute form as winter washes for fruit trees. They have been tested as wound protectives both in the concentrated form and at a dilution of about 6 per cent., the strength at which they are employed for spraying. In either form they are much too liquid to give any mechanical protection, while any lethal properties they may possess appear to have no adverse effect on the mycelium of *Stereum purpureum.*

Home-made Red Oxide Paint. This paint, which had previously been fairly successful as a wound protective, gave disappointing results in the winters of 1925-26 and 1926-27, and its use has been abandoned.

Bordeaux Mixture Paint. This is made by adding sufficient linseed oil to Bordeaux Mixture powder to give a paint of the consistency required for application to tree-wounds. Unless considerable care is taken, an uneven paint results. It also needs thorough stirring before use. When applied thickly it gives a good surface. The paint cracks and flakes badly with the lapse of time, but this is probably not a serious objection, since the most important period for wound protection is that immediately after exposure. Very large quantities of gum frequently exude from the margins of wounds protected with this paint, possibly in reaction to the copper contained therein. This gum sometimes lifts the paint surface bodily away from the wound ; in other cases it bursts through the paint.

Callus is formed less readily beneath this paint than beneath Soft Grafting Wax and White Lead Paint. As a wound protective Bordeaux Paint has proved fairly efficient, though on the whole less so than the two protectives described below.

Home-made White Lead Paint. Further experiments with this paint confirm the results previously obtained; apart from a very occasional failure it has continued to prove an excellent protective against *Stereum purpureum.* It is easily applied with a brush to wounds of any size, it has little or no toxic effect on the bark even of young shoots, it gives a good and lasting surface and does not inhibit the formation of callus. It is the best protective for large-scale use in orchards, where the difficulty of applying Soft Grafting Wax makes the use of this substance impracticable. The formula according to which this home-made paint is made up was given in " Silver-leaf Disease, V " (2).

Soft Grafting Wax. The good results previously obtained with this substance have been fully confirmed by more recent work. Apart from the difficulty of applying it to very large wounds or to large numbers of wounds, Soft Grafting Wax is the ideal protective. If applied in reasonable quantity it forms a surface which remains intact and almost impermeable until it is lifted off the wound by the developing callus. Moreover, wounds protected with this wax form callus more readily than those protected with other substances. It has no toxic effects whatever. It is the only protective, apart from Anti-fouling Paint, which has not failed rather badly at least once during the trials. For nursery work, or whenever the number of wounds is comparatively small and the wounds themselves not too large, Soft Grafting Wax should be used in preference to any other protective.

III. SUSCEPTIBILITY OF DERIVATIVES OF " SELFED " VICTORIA PLUMS TO SILVER-LEAF DISEASE.

Some years ago Victoria plums were " selfed " at the John Innes Horticultural Institution. The derivatives varied greatly in habit and in leaf and fruit characters, and it was thought desirable to test a selection of the various types for susceptibility to Silver-leaf Disease. For this purpose the selected types were budded on a uniform stock (Common Mussel), obtained from the East Malling Research Station, and when they had grown sufficiently were inoculated with *Stereum purpureum.* Records were taken of the incidence of silvering and of the amount of recovery therefrom in subsequent years.

The fifteen types of trees experimented with showed considerable differences in susceptibility to infection by *Stereum purpureum* and also in their tendency to recovery after infection. Some of these types of trees became infected to an extent of 100 per cent. of the inoculations made, while a few types showed a considerable degree of resistance to infection, which was also correlated with a high rate of recovery from silvering.

The method of experimentation was as follows: in March of each of the years 1927, 1928 and 1929, branches about ¼ to ½ inch in diameter were cut back on certain of the trees and the wounds thus made were inoculated twice within the next few days with a spore-suspension of *Stereum purpureum*. Records of the results of the inoculations were taken every summer. Unfortunately, owing to the small number of trees available, some of the branches inoculated in 1927 had to be removed in the autumn of 1928 to make room for further inoculations on the same trees. Few of the inoculations, therefore, were under observation for the whole four years during which the experiment was in progress, while for some inoculations records for only two years are available.

In the figures given below, which concern a small selection of the types of trees experimented with, no account has been taken of the actual years in which the inoculations were made. As an example, those on the trees the type-number of which was 143, may be considered. There were six trees of this type available for the experiment. In March, 1927, twelve wounds were cut on four of these trees—143 (ii), 143 (iii), 143 (iv) and 143 (v). In March, 1928, ten wounds were cut on two other trees—143 (vi) and 143 (vii). In the autumn of 1928 the branches bearing the first twelve wounds were removed from the trees (care being taken to cut back to clean wood), and in March, 1929, they were again used for inoculation after re-cutting, thirty-one additional wounds being made on the four trees. The percentage of successful infections has been calculated for the first, second and third seasons after inoculation. The percentage for the first season is based on the 1927 summer records for the first twelve wounds, on the 1928 summer records for the second ten wounds, and on the 1929 summer records for the thirty-one wounds which replaced the first twelve. The percentage for the second season is similarly calculated. The only wounds for which a third season's record is available are, of course, those cut in 1928.

In all, 470 wounds on these derivatives of selfed Victorias were cut and inoculated. In the first summer after inoculation 323 of these (69 per cent.) became silvered. By the second year some recovery had taken place; the number of wounds still silvered was then 199 (42 per cent.).

The wounds for which a three-year series of records is available number 275. Of these, 202 (73 per cent.) were silvered in the first, 178 (65 per cent.) in the second, and 160 (58 per cent.) in the third summer after inoculation. There was, therefore, a steady decrease in the total amount of silvering on these trees in the years following the inoculation of the wounds.

The three types of trees which were most promising as regards resistance to, and recovery from, the disease were those numbered 143, 313 and $2\frac{6}{10}$. The figures for these are included in the table below, calculated both for the first two seasons after inoculation (including all the wounds cut) and for the first three seasons, for which, unfortunately, only a minority of the wounds are available. For comparison, the figures for three of the more susceptible types are also included.

Type No.	Records over two years.				Records over three years.				
	Number of wounds inoculated.	Percentage Infection in First season.	Percentage Infection in Second season.	Percentage Recovery.	Number of wounds inoculated.	Percentage Infection in First season.	Percentage Infection in Second season.	Percentage Infection in Third season.	Percentage Recovery in Third season.
143 ..	53	40	11	71	10	60	20	0	100
313 ..	63	25	8	69	16	31	6	12	60
2_{10}^{6} ..	57	54	16	71	16	12	0	0	100
260 ..	25	100	92	8	10	100	90	90	10
303A ..	15	87	67	23	12	83	58	42	50
304 ..	21	90	100	—	12	100	100	100	0
All Types together ..	470	69	42	38	275	73	65	58	21

It will be seen that the three types 143, 313 and 2_{10}^{6} are well above the average both as regards resistance to infection by *Stereum purpureum* and as regards recovery from infections which became established. Of the three types, the first two (143 and 313) appeared the most satisfactory in respect of habit, etc., and a large number of Common Plum stocks have now been budded with these two types so that further tests can be made.

IV. SUSCEPTIBILITY OF VICTORIA PLUMS WORKED ON VARIOUS STOCKS.

Experiments were carried out at Melbourn in 1926 and 1927 to investigate the possible influence of the nature of the stock on the susceptibility to Silver-leaf Disease of the scion worked upon it. The trees used were young, vigorous Victoria plums, obtained from the East Malling Research Station, and worked on ten different stocks. Pruning wounds were cut on the trees early in April in 1926 and 1927 and were inoculated at once with a spore-suspension of *Stereum purpureum*. Records of the incidence of silvering were taken during the summer following inoculation. Only the results in the summer following inoculation are given in the following table, as serious attacks of " Die-back " in the plantation prevented any investigation of the relative powers of recovery from Silver-leaf Disease of the different types.

Victoria on	1926.			1927.			1926+1927.		
	No. of inoculations.	No. of infections.	Per cent. infections.	No. of inoculations.	No. of infections.	Per cent. infections.	No. of inoculations.	No. of infections.	Per cent. infections.
Myrobolan A. ..	20	18	90	22	10	45	42	28	67
Myrobolan B. ..	20	20	100	30	2	7	50	22	44
Myrobolan C. ..	26	22	84	16	3	19	42	25	60
Broad-leaved Mussel ..	17	11	65	24	2	8	41	13	32
Common Mussel	12	9	75	17	1	6	29	10	34
Brussels	13	8	62	16	0	0	29	8	28
Brompton ..	23	14	61	16	1	6	39	15	38
Pershore	11	10	91	29	6	21	40	16	40
Marianna ..	26	14	54	24	5	21	50	19	38
Common Plum ..	26	24	92	29	1	3½	55	25	45

No very considerable differences in susceptibility are evident in the above table, although trees on Myrobolan A appear to be relatively most susceptible and those on Brussels most resistant. The variation in the number of successful infections for a particular stock in the two years is noteworthy. It is obvious that experiments of this kind would have to be carried out over a long period of years before conclusions could be safely drawn from them.

As pointed out in " Silver-leaf Disease, IV " (1), there is some field evidence that the nature of the stock may influence the susceptibility of the Victoria plum to the disease. This influence, however, probably operates in a practical way only when the trees are approaching maturity, and then through its effect on the habit of the tree and on the liability of the branches to break.

V. GUM-BARRIERS.

In previous papers of this series (1, 2) accounts were given of the gum-barriers which are associated with recovery from attack by *Stereum purpureum* or which inhibit infection by the fungus. Most of the results described in those papers were obtained by cutting pruning wounds on small branches of plum trees, inoculating the wounds thus made with a spore-suspension of *Stereum purpureum*, and examining the infected twigs after varying periods of time. Since 1925 further experiments on these lines have been carried out and the work has been extended with the object of determining what occurs beneath similar wounds which are not inoculated with *Stereum purpureum*. The results of these experiments show that gum-barriers are formed at certain times of the year beneath pruning wounds quite apart from invasion by *Stereum purpureum*. These results agree closely with those obtained by Swarbrick (3).

Before proceeding further it is necessary to point out an extension in the use of the term " gum-barrier." In " Silver-leaf Disease," IV and V (1, 2) the term is reserved for the blackened zones, characterized by very heavy gumming in the vessels of the wood, which enclose the fungus *Stereum purpureum* in trees which have been unsuccessfully invaded, and in those which have recovered from the disease. In view of the more recent work described below it seems desirable to extend the meaning of the term "gum-barrier" to include all such heavily-gummed and blackened zones whether they are formed in association with an invasion by *Stereum purpureum* or not.

A. GUM-FORMATION BELOW UNINOCULATED WOUNDS.

In dealing with very large numbers of wounds in the field it has been impossible to take precautions to prevent their invasion by fungi, and such invasions have frequently occurred. A few wounds, however, were carefully protected against fungal invasion and these gave results similar to those not so protected. It seems probable that, although invading fungi, the majority of which are saprophytes, may modify the times and the distances from the surface at which gum-barriers are laid down, the method of gum-barrier formation is essentially the same whether these fungi are present or not. A few of the wounds were invaded by vigorous parasites, including occasionally *Stereum purpureum*, but wounds invaded in this way were eliminated from the experiments. A further reference to the question of invasion of these uninoculated wounds will be found below.

The method of experimentation was as follows: pruning wounds were cut on plum branches about ¼ to ½ inch in diameter, the cut being usually made close above a lateral shoot. These wounds were cut at monthly intervals throughout the year, and the formation of the gum-barriers was followed by examining the twigs after varying periods of time. Most of the work was done on the Victoria and Czar varieties, both of which are susceptible to Silver-leaf Disease, but a smaller experiment on the resistant Pershore variety was also included. The results obtained with these uninoculated wounds on the Victoria and Czar varieties will be considered first.

The first change in the twig after wounding, as seen macroscopically, is a slight browning of the wood immediately below the cut surface. The time which elapses between the cutting of the wound and the appearance of this browning varies according to the period of the year at which the experiment is performed, being very much shorter during the summer than at other times. The next stage is the appearance of a darker brown discoloration, which is usually first seen in the outermost wood near the cambium. Later, the base of the more central light brown discoloration also shows this darkening. The depth of the first-formed light brown zone varies considerably, but it is hardly ever less than ¼ inch and may reach as much as 2 inches. The very large number of wounds, cut at all periods of the year, in which the depth of this light brown zone is ¼ to

¾ inch suggests that possibly this is the depth which is characteristic of uninvaded wounds, and that the depths of 1 to 2 inches sometimes found are usually to be associated with invasion of the wounds by weak parasites. A diagrammatic representation of the shallow and extensive types of discoloration is given in Figs. 1 and 2.

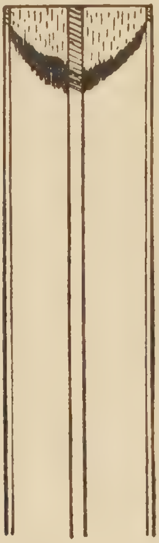

FIG. 1.
(Nat. size.)

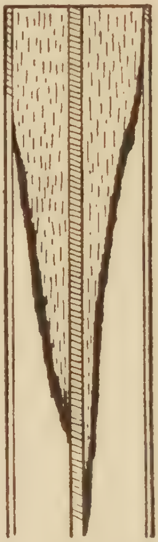

FIG. 2.
(Nat. size.)

It will be obvious that no real distinction can be drawn between the " sides " and the " base " of most of these discoloured tissues. But for convenience such a distinction may be drawn and the terms " lateral " and " terminal " will be employed. The darker brown type of discoloration is never seen immediately below the cut surface although it may approach to within ⅛ inch of it, especially in the outer wood. The appearance of this darker type of discoloration results in the first-formed central light brown zone being bounded, laterally and terminally, by a rather narrow darker zone. The final stage in the formation of a gum-barrier is a progressive darkening, and sometimes also a thickening, of this already somewhat dark zone. A completed gum-barrier is usually almost black. This blackening, like the darkening which precedes it, is usually first seen in the lateral regions.

Microscopical examination shows that in the first-formed light brown zone the gum-like substances, to which the discoloration of the wood is due, are mostly in the medullary ray cells although they may occur in small quantities at the septa or lining the walls of some of the vessels. Later, as stated above, a darker type of discoloration appears at the edges of the light brown zone. This darker brown colour is associated with changes both in the quantity of gum in the tissues and in its distribution. In these darker zones the gumming is decidedly heavier than in the lighter areas, and the great mass of it is in the lumina of the vessels and not in the medullary rays. As far as can be seen microscopically, a completed and blackened gum-barrier differs from the dark brown lateral or terminal regions just described only in a greater concentration of the gum in the vessels. It may be, however, that differences in the chemical constitution of the gum in the two regions also exist. In a barrier zone the medullary ray cells are sometimes, but by no means always, devoid of gum.

Although the sequence described above seems always to be followed, the time which elapses before the gum-barrier is completed varies greatly according to the season at which the wounds are cut. Vessel-gum never seems to be laid down in any quantity before April or after October, while its formation is much more rapid and considerable in quantity in June, July and August than in other months. Wounds cut, for instance, in November will perhaps show the first macroscopic signs of discoloration of the wood in February. This slightly discoloured area will increase in depth very slowly in the following months until, perhaps in April or May, a slight darkening, first laterally and then terminally, announces that gum-barrier formation is beginning. This newly-formed terminal zone will increase in darkness and to some extent, perhaps, in thickness, until by July or August a fairly thick and almost black gum-barrier has been laid down. Little change will take place after this even if the twigs are left on the tree for two or more years. The available evidence suggests that in succeeding years the blackened barrier zone sometimes increases in thickness, but whether this increase is at the expense of the upper light brown zone or at that of the healthy wood below the barrier it is impossible to say. But even after a single summer the blackened area may be of considerable thickness; it often accounts for one-third or even one-half of the total depth of discoloration. Wounds cut in the summer months (May-August) show the same series of changes, but they follow one another more quickly. Even in these months, however, a blackened barrier zone is very rarely seen until at least two months after the cutting of the wound. The actual time taken probably depends to some extent on external factors such as temperature and humidity, although no systematic attempt has been made to prove such a correlation. Weather conditions probably help to decide whether or not wounds cut in August and September will be blocked before the following winter. Barrier-formation usually seems to require at least two months, and the constituent vessel-gum is rarely laid down in quantity after October. If weather conditions in September and October favour gum-formation, wounds cut in August will probably complete their gum-barriers during the two following months, while those cut in September may show at least the first stages in the formation of a barrier. Under unfavourable conditions wounds cut in September will probably remain without a barrier until the following summer and will thus resemble wounds cut during and after October.

There were no observable differences between the Victoria and Czar varieties, either as regards the time of formation of the gum-barriers or as regards their positions relative to the wounds.

Similar experiments with uninoculated wounds were also carried out on the resistant Pershore variety. Here the sequence of changes leading to the formation of a gum-barrier was found to be exactly the same as in the Victoria and Czar varieties. But whereas, in the latter, the first signs of gum-barrier formation were not seen before April, in Pershore these signs were occasionally seen in March. The barriers, too, were formed nearer the cut surface in the Pershore twigs, the great majority being found within ½ inch of the wound. On the whole, too, at the end of a single season, the thickness of the barriers relative to the total depth of discoloration was greater in the Pershore twigs than in those of Victoria and Czar. The resistant variety seems, therefore, to form gum-barriers (in the absence of *Stereum purpureum*) rather more readily than do the two susceptible varieties tested, but no very striking differences are to be observed.

B. GUM-FORMATION BELOW WOUNDS INOCULATED WITH SPORES OF *Stereum purpureum*.

Monthly experiments, similar to those with uninoculated wounds described in section A, except that the exposures were immediately inoculated with a spore-suspension of *Stereum purpureum*, were carried out on trees of the Victoria and Czar varieties; the inoculated twigs were examined at intervals in order that the progress of the infections might be followed. Additional information on the subject of gum-barriers was obtained by the examination of trees which had recovered from Silver-leaf Disease.

As with the uninoculated wounds, vessel-gum is never formed in any quantity between October and April, while gum-barriers are laid down only during the summer months. Except when the inoculations were made during the summer, a high percentage of the inoculated twigs subsequently became silvered. The silvering of the foliage was normally associated with discoloration of the wood to a considerable depth below the wound. In general the results obtained with these inoculated wounds for any one month did not show anything like uniformity as regards the progress of infection. Only those cut and inoculated in the months of June and July showed any approximation to constancy in this respect. The great majority of the twigs were left on the trees until the late summer or autumn after the inoculations were made—i.e. until the host had had the chance to form gum-barriers. The wounds for a given month were all made on approximately similar branches of a single large tree; they were all cut at the same time and were all inoculated with the same spore-suspension. In months other than June and July the differences between individual inoculated twigs were often very great. In some branches, by the autumn after inoculation, the fungus might have been stopped by a gum-barrier within a few inches of the inoculated wound; this comparatively limited zone of discoloration might or might not be accompanied by silvering of the foliage. From other inoculated branches the fungus might have spread into the trunk of the tree; these extensive discolorations were usually accompanied by silvering symptoms. Such great variations in the results obtained with inoculations made in any one month render insignificant the differences between inoculations made in different months of the year other than June and July, when the inoculated wounds show sufficiently constant results to form an exception to this statement. In twigs inoculated in these months the fungus seldom makes much progress before it is stopped by a gum-barrier. In fact, it is often impossible to distinguish macroscopically wounds inoculated in these months from the corresponding uninoculated wounds.

The various results obtained with wounds cut and inoculated in months other than June or July may usefully be summarized as follows:—

(1) The fungus is not stopped by a gum-barrier during the first summer after inoculation, but continues to spread down the branch. Such infections are usually, but not always, accompanied by silvering of the foliage. Those branches silvered in the first season, during which no gum-barrier was laid down, normally show the silvering symptoms in the second season also, while those which were externally healthy in the first season sometimes show silvering in the second. It would seem that in these last-mentioned cases the conditions during the first summer must have been unfavourable both for the spread of the fungus and for the formation of vessel-gum by the tree. If the fungus had been growing vigorously it would probably have caused silvering of the foliage, while weak growth of the fungus, under conditions favourable to gum-formation, would probably have resulted in the formation of a gum-barrier. Branches silvered in both of the first two seasons, or in the second season only, may or may not become healthy in the third or in any subsequent season. When such recovery takes place it is normally associated with the formation of a gum-barrier in the summer before that in which the branch again bears healthy foliage.

(2) The fungus progresses some distance down the branch but is stopped by a gum-barrier during the summer following inoculation. The extent of invasion varies considerably in different branches. Silvering of the foliage may or may not appear in the summer in connection with the infection. The factors which determine whether or not the fungus will cause silvering are obscure; it is probable, however, that the vigour of the fungus at the time when the leaves are unfolding is of primary importance. Branches in which the growth of the

fungus has thus been stopped usually show no silvering in the second summer after inoculation. That the recovery of these branches can usually be associated with the presence of gum-barriers formed in the first season is established by examining such recovered branches in the second May after inoculation—i.e. before any appreciable gum-formation has occurred in that year. Any gum-barrier then found can safely be ascribed to the preceding summer, i.e. the first after inoculation.

At whatever stage an infected but still living branch is examined, the zone of discoloration due to the disease is seen to be darker at its margin (both lateral and terminal) than in the centre. The degree of this marginal darkening varies considerably according to the progress which the fungus is making. The different cases may be summarized, somewhat schematically, as follows:

(1) The margin of the invaded tissues is somewhat darker than the central zone. Here the fungus is probably still making progress both laterally and longitudinally. It should be noted that lateral extension is always slow compared with longitudinal extension, partly owing to the greater number of cell-membranes which the fungus has to traverse.

(2) The lateral part of the margin is black but the terminal part is still brown, although somewhat darker than the central zone. The lateral progress of the fungus has been stopped by a gum-barrier; longitudinal extension is still continuing.

(3) The margin is wholly black. Here both lateral and longitudinal progress of the fungus has been stopped by a gum-barrier, and the fungus is completely occluded.

Intermediate cases occur, and it is often impossible to determine whether the more or less blackened marginal zone is sufficiently well developed to stop the progress of the fungus—in other words, whether a gum-barrier has been formed or not. Sometimes, too, when the fungus is advancing rapidly down the branch there is no discernible difference in colour between the extreme base of the discoloration and the older central part. In general, however, the reactions of the invaded tissues can be ascribed to one of the three classes described above. It is hardly necessary to say that classes (1) and (2) represent only stages of invasion and not a final state such as is indicated in class (3). Had they been left for a longer period before examination the branches which in fact showed stage (1) might have given very different results. If fungal growth had continued unchecked it would eventually have resulted in the death of the branch. Under other conditions, however, the branches might, after a longer period, have shown either stage (2) or stage (3).

It will be noticed that the different zones of the invaded tissues correspond to those in the discoloured region below uninoculated wounds; they are due to the same causes—differences in amount and distribution of the gum present in the cells. It will be unnecessary, therefore, to describe in detail the microscopical appearance of these different zones. As has been indicated in previous papers of this series (1, 2), the mycelium of the fungus is normally found only in the central zone.

In the absence of fungi, however, the tissues below a wound usually pass through the whole sequence of changes culminating in the formation of a complete gum-barrier ; moreover, the reactions are usually localized within an inch or so of the surface and take place within a year of the cutting of the wound. But when an active parasite such as *Stereum purpureum* is present, gum-barrier formation may never occur at all, or it may take place from half an inch to several feet below the surface within a period possibly extending over several years from the beginning of the invasion.

As has been stated above, the discoloured zone associated with an infection by *Stereum purpureum* can nearly always be divided into two parts, a central comparatively light brown region and a narrow darker marginal one. Since this is so at whatever stage in the invasion a branch is examined, it follows that as the hyphæ advance, either laterally or longitudinally, there must be a continuous partial transformation of the gum in the region which the mycelium is entering, together with fresh gum-formation a short distance further ahead of the hyphæ. This partial transformation of the gum, especially vessel-gum, may be due either to some spontaneous change in the nature of the gum with the lapse of time or to the presence of the fungus. In the absence of evidence that such transformation of newly formed vessel-gum takes place in branches uninvaded by fungi it seems probable that it is to be ascribed to the action of the hyphæ.

It will now be convenient to attempt a description of the possible mechanism of gum-barrier formation in association with invasion by *Stereum purpureum* ; for simplicity it will be assumed that the infection has proceeded so far down the branch that gum-formation as a direct wound-response can be neglected. Two factors which must largely influence the formation of gum-barriers in association with an attack of this kind are (1) that vessel-gum is formed more readily in the younger wood near the cambium than in the older tissues, and (2) that *Stereum purpureum* in a branch spreads much more rapidly in a longitudinal than in a lateral direction. The hyphæ advance slowly in a lateral direction towards the cambium. The healthy wood just beyond the mycelium is stimulated to produce gum, first in the medullary ray cells and then in the vessels. The nearer the fungus is to the cambium the more freely is gum formed. At the same time the hyphæ spread rapidly downwards in the vessels. Again, the wood just in advance of the hyphæ is stimulated to form gum, first in the medullary ray cells and then in the vessels. In general this gum-reaction will not be so vigorous as that in front of the laterally advancing hyphæ, since it is the more central part of the wood which is for the most part affected. Moreover, before sufficient time has elapsed for much gum to be formed the rapidly advancing hyphæ will have reached the reacting zone and will have begun to transform some of the gum already present. Meanwhile, the wood a little lower down the branch will in turn have been stimulated and will be producing gum. This process may be repeated indefinitely, the wood a short distance ahead of the advancing hyphæ being always somewhat darker than the tissues containing the mycelium.

If the fungus is growing very vigorously or if the host is weak—i.e. if the balance between host and parasite is much in favour of the latter—the fungus will probably make headway both in a lateral and a longitudinal direction. The slower lateral progress is due partly to the larger number of membranes which the fungus has to traverse and partly to the greater gum-formation in the younger wood. If the balance between host and parasite is slightly more favourable to the host it is probable that the heavier gum-formation in the younger wood will actually prevent any further lateral spread by the fungus, even if gum-formation in the more central wood at the base of the infection is still insufficient to check the downward progress. This accords with the fact that, even when the fungal attack is ultimately entirely successful, a long period elapses before the branch begins to die back—i.e. before the fungus has reached and killed the cambium. It is frequently found, too, that the fungus may spread downwards from an inoculated branch into the trunk of a tree and even into the roots, and yet may never reach the cambium at all. Of course, this does not prove that the fungus has not been making very slow lateral progress throughout the duration of the infection, since it is clear that in order that the cambium may be reached by the fungus, the rate of lateral spread by the latter must be greater than the rate at which the cambium is laying down new wood. The extremely long and narrow discolorations often found suggest, however, that lateral progress by the fungus is frequently not only much retarded but stopped altogether, while rapid longitudinal extension still continues.

After the fungus has spread some distance down the branch in the manner described it may happen that the host-parasite relation is altered very much in favour of the host. The result will be the same whether this alteration is due to conditions which cause more ready gum-formation in the host, or to those which depress the rate of growth of the fungus. In the first case the wood in advance of the fungus will produce more gum in a given time ; in the second a longer time will be available for gum-formation before the fungus reaches the reacting zone. In either case the probable result will be that so much gum is laid down that the fungus cannot penetrate it. In other words, the fungus is now completely enclosed by a gum-barrier. Within this barrier the fungus may continue to live for a considerable time—it has been obtained by plating out about a year after its enclosure—but eventually it dies, presumably because it has exhausted all the food supplies within the barrier. It is probable, too, that "staling products" accelerate the death of the mycelium.

VI. MANURIAL EXPERIMENTS IN CONNECTION WITH SILVER-LEAF DISEASE.

In "Silver-leaf Disease, V" (2) three manurial trials were described and the results obtained until 1925 were given. These experiments aimed at increasing the amount of natural recovery from the disease by enhancing the vigour of the trees. Two of the trials were carried out at Melbourn, the other in Worcestershire. The two Melbourn experiments have been continued; in addition two other manurial experiments have been instituted since 1925—a small trial at Melbourn and a larger one in Worcestershire.

In 1923 a manurial experiment was commenced at Melbourn. The trees were Czar plums about fifteen years old and growing in grass. They were on good land and of fairly vigorous growth. Groups of ten of these trees were treated with kainit, basic slag and sulphate of ammonia, either separately or in combination, at the rate of 4 lbs. each per tree, two applications being given in December and March in each of the years 1922-3, 1923-4 and 1924-5. Since 1925 no manure has been applied to these trees but yearly records have been taken of the amount of Silver-leaf Disease present. The following table shows the results for the first two years of the experiment (1924 and 1925) and for the last two (1929 and 1930). It is desirable to repeat the 1924 and 1925 figures although they are substantially those published in "Silver-leaf Disease, V," since they have since been slightly modified by the removal of a few of the trees from the experiment. The figures are calculated on the "point system" described in "Silver-leaf Disease, V" according to which marks are assigned to the trees on the following basis:

Healthy	0
Trace of silvering	1
Slight silvering	2
Moderate silvering	3
Heavy silvering	4
Killed by Silver-leaf Disease	5

It will be seen that with some of the treatments the amount of recovery has been very great, notably with kainit+basic slag+sulphate of ammonia, with basic slag+sulphate of ammonia and with kainit+basic slag. Since 1925, when the application of manures ceased, the trees have shown well marked yearly fluctuations in the amount of Silver-leaf present; with two exceptions,

Treatment.	No. of trees which have totally recovered since 1925.				No. of Trees dead in 1930.	Percentage Recovery on Point System.			
	1924.	1925.	1929.	1930.		1924.	1925.	1929.	1930.
Kainit, Basic Slag, Sulphate of Ammonia (10 trees)	3	7	9	9	0	45	76	94	97
Kainit, Basic Slag (10 trees)	4	6	7	9	0	64	82	79	88
Kainit (10 trees) ..	4	6	5	6	1	48	58	55	61
Kainit, Sulphate of Ammonia (9 trees)	1	4	4	4	1	10	38	45	45
Basic Slag, Sulphate of Ammonia (10 trees)	6	6	9	9	0	73	61	91	91
Basic Slag (10 trees)	3	4	6	7	1	44	56	62	71
Sulphate of Ammonia (10 trees)	3	3	3	6	3	21	24	27	45
No Treatment (40 trees)	14	17	25	27	4	45	56	65	66

which are mentioned below, the variously treated groups of trees show remarkable similarities in these yearly fluctuations. For example, in 1926 there was a marked increase of silvering in nearly all the groups of trees ; in 1927 there was a great general decrease in silvering, followed by another rise in 1928 and by further decreases in 1929 and 1930. The groups of trees treated with two of the most successful manurial mixtures (kainit+basic slag+sulphate of ammonia and kainit+basic slag) do not, however, show these fluctuations, for since 1925 both these groups have shown a steady decrease in the amount of silvering present.

Another manurial experiment started at Melbourn in 1923 was carried out on Czars of about the same age as those in the trial described above, but weaker in growth and growing in clean cultivation on poorer soil. The results obtained up to 1925 with the various manurial mixtures tried were given in " Silver-leaf Disease, V " ; many of the trees were then dead and the great majority of the remainder were silvered. No manures were applied to these trees after the spring of 1926. Since 1925 there has been, on the whole, some improvement in the condition of the surviving trees, although those groups treated only with kainit or with basic slag have continued to show deterioration. The most satisfactory substance used in this trial was the mixture of kainit+basic slag ; the trees thus treated show a recovery in 1930 of 11 per cent. on the point system, while the control trees show a deterioration of 25 per cent. It will be noted that these figures compare very unfavourably with those obtained in the experiment with Czars growing in grass. This is largely to be attributed to the poorer soil and to the weaker growth of the trees ; many of them were further weakened by severe attacks of Brown Rot. One interesting feature has emerged from this experiment. By far the greater number of trees which died from the disease did so in the first few years of the trial ; the survivors, however, have in general improved considerably. This is well shown in the group of trees treated with kainit + basic slag and in the control group. Fifteen silvered trees were treated with kainit + basic slag from 1923 until 1926 ; eight of these were dead by 1924. Since then there have been no further deaths and by 1930 six of the seven survivors had become healthy, while the seventh showed only traces of silvering. Of the seventeen silvered control trees ten were dead by 1925. By 1927 three others were dead but the four survivors were all healthy in 1930. It seems comparatively unusual for a tree to be silvered for a period of several years and then to die from the disease. If it does not succumb in the first few years it has quite a good chance of recovery.

A small manurial experiment was started at Melbourn in 1926 to test the effect of a mixture of kainit, superphosphate and farmyard manure. The trees used were Czars growing in clean cultivation in the same plantation as those used in the larger manurial trial just described. Most of them had become silvered a year or two previous to the commencement of the trial as a result of inoculation experiments. 2 lbs. kainit and 1 lb. superphosphate were applied to each of the trees in January and April, 1926, and in January, 1927, while a good dressing of farmyard manure was given in March, 1926. Twenty-one trees, all silvered in 1925, were treated in this way and twenty-one similarly silvered trees were left untreated as a control. By 1930 twelve of the manured trees had become healthy and the improvement on the point system of this group of twenty-one trees was 35 per cent. In the same period five of the twenty-one control trees became healthy but the group showed a deterioration of 35 per cent. on the point system. In 1930 five of the treated and fourteen of the control trees were dead and it is noteworthy that all of these were killed by the disease between 1925 and 1927. These results, therefore, confirm those of the larger experiment in that the trees which survived the first few years of attack by the disease subsequently showed very considerable improvement. The most striking difference between the treated and the control trees lies in the much larger number of treated trees which were able to remain alive through the critical period.

The second of the new manurial experiments was started in the autumn of 1926 in a fruit plantation belonging to Mr. C. Vint at Drakes Broughton in Worcestershire. This trial was carried out in collaboration with Mr. T. Wallace of the Long Ashton Research Station and Mr. Gaut, Agricultural Organizer for Worcestershire. Most of the silvered trees were Victoria plums, about thirty-five years old, growing vigorously in clean cultivation on a rather heavy loam. It was desired to use a manurial mixture which would promote the most vigorous growth unaccompanied by succulency. The soil was analysed at the Long

Ashton Research Station and on the basis of this analysis Mr. Wallace advised the following manurial treatment: during the winters of 1926-7, 1927-8 and 1928-9 farmyard manure was to be ploughed in at the rate of 10 tons per acre; this was to be supplemented by an application of 1½ cwts. of steamed bone flour (60 per cent. total phosphates grade) per acre. The manures were applied at these rates in the winter of 1926-7; in the two following years, however, the farmyard manure was increased to 15 tons per acre, the steamed bone flour remaining as before. This experimental treatment of the trees was not continued after the winter of 1928-9. In the late spring of 1929, however, in view of the approaching end of the experiment, the grower wished to assist the hitherto unmanured (control) trees by treatment with Peruvian guano. In order that the experiment should not be unnecessarily interfered with this dressing was applied to all the trees, both those previously experimentally treated and the controls. A similar dressing of Peruvian guano was given to all the trees in 1930.

During the period of the experiment (1926-1930) records were taken twice yearly of the amount of silvering on the trees. We are indebted to Miss R. Collett, Inspector of the Ministry of Agriculture, for help in taking these records. Yearly records of the vigour of the trees were taken by Mr. Wallace and Mr. Gaut.

The results obtained are summarized in the following table:

Treatment.	No. of trees which have totally recovered since 1926.				No. of trees dead in 1930.	Percentage Recovery on Point System.			
	1927.	1928.	1929.	1930.		1927.	1928.	1929.	1930.
Farmyard Manure + Steamed Bone Flour (35 trees)	11	16	19	26	1	18	32	34	65
No Treatment (23 trees)	9	9	14	17	1	28	33	39	71

It will be noted that there has been a considerable amount of recovery from the disease since the beginning of the experiment but that there is no significant difference in this respect between the manured trees and the controls.

Neither of the two groups of trees (manured or control), taken as a whole, has shown any significant change in vigour during the period of the experiment, although there has been considerable improvement in both with respect to Silver-leaf Disease. It is possible, however, to show that some correlation exists between vigour and recovery from the disease, as is indicated by the following results:

Ten trees, originally silvered, showed a marked increase in vigour during the period 1926-30. These trees showed recovery from Silver-leaf Disease amounting to 95 per cent. on the point system.

Forty trees, originally silvered, remained unchanged as regards vigour. The recovery from the disease in these trees amounts to 76 per cent.

Six of the trees originally silvered have shown a marked decrease in vigour. The intensity of Silver-leaf Disease in these trees has increased by 12 per cent. during the period 1926-30. This last result, although suggestive, is not very significant because of the small number of trees concerned. Moreover, four of the six trees actually became healthy during the period under consideration although these four had never been more than very slightly silvered.

In this attempt to correlate recovery from Silver-leaf Disease with vigour no distinction has been made between the manured and the control trees. If, however, these are treated separately, essentially similar results are obtained.

In considering the results of these manurial experiments as a whole there does not appear to be any special treatment of this kind which can be universally recommended to facilitate recovery from Silver-leaf Disease. There is no doubt that when silvered trees are growing vigorously they will tend to recover more from the disease than when they are growing weakly. Appropriate manurial treatment to stimulate vigour will vary according to the nature of the soil on which the trees are growing, but in our own limited experience phosphatic and potassic manures have been found to be most beneficial in this respect.

VII. SILVER-LEAF DISEASE IN PLUM STOCKS AND YOUNG NURSERY TREES.

During the last three years a considerable amount of work has been done in connection with the incidence of Silver-leaf Disease in plum stocks and young nursery trees, with the object of determining as far as possible the usual sources of infection in such trees. Growers sometimes complain that young fruit trees, apparently healthy when they are received from the nursery, develop the disease a few years after they have been planted out. It has been suggested that these outbreaks of Silver-leaf Disease are due to infections which took place while the trees were still in the nursery, but that the disease remained " latent " until after re-planting. To investigate the possibility of this, numbers of budded nursery trees of several varieties of plum and peach have been obtained from various sources and have been grown at Cambridge for several years. It is also said that silvered plum suckers are sometimes used as stocks in nurseries and that the disease is thereby transmitted to the trees. Large numbers of such suckers have been examined to ascertain whether they actually contain the mycelium of *Stereum purpureum*, while many others have been planted out at Cambridge so that the course of the silvering could be followed in succeeding years.

A. EXAMINATION OF SILVERED PLUM SUCKERS.

Several heavily silvered Victoria plum trees, from the root systems of which silvered suckers had arisen, have been examined in some detail, the roots being traced from the tree to the suckers and examined for the mycelium of *Stereum purpureum*. Although the discoloration associated with the presence of the fungus often extended some distance into the roots, it was found that the great majority of the suckers arose from the clean parts of the roots into which the mycelium had not spread. Silvered suckers were frequently found at considerable distances from the parent trees ; one such sucker was 18 feet from the tree and at least 16 feet beyond the nearest discoloration due to *Stereum purpureum*. In view of what has long been known about the relative distribution of the silvering symptoms and the mycelium in the aerial parts of silvered trees this, however, is not surprising.

Group I. Suckers from Hook (Hants).

In July, 1929, our attention was drawn to some Victoria plum trees in a plantation at Hook (Hants) which had been severely attacked by Silver-leaf Disease and which had been cut back to within a few feet of the ground. Numerous suckers, most of them silvered, had sprung from the roots of these trees. This afforded an opportunity of obtaining for subsequent observation suckers whose condition before separation from the parent trees could be fairly accurately determined, together with information as to the presence or absence of living mycelium of *Stereum purpureum* in the roots. The suckers from eight of these trees were therefore labelled and recorded for silvering in July, 1929. In the following November the trees were grubbed in such a way that the roots were damaged as little as possible. The position of each group of suckers and the condition of the root from which it arose was noted. The suckers were then detached and planted at the University Farm. They have been kept under observation during the summer of 1930 and those still living will be allowed to remain for a further period.

Stereum purpureum had entered many of the roots. Some of the suckers sprang from those parts of the roots which were actually discoloured by the fungus, but the majority arose on parts of the roots into which the mycelium had not spread. The distance of some of these silvered suckers from the nearest discoloration due to *Stereum purpureum* was considerable.

A number of the suckers planted out at Cambridge in November, 1929, died during the winter or during the early summer of 1930, apparently through lack of an adequate root-system. These deaths from causes unconnected with Silver-leaf Disease have made impossible a complete analysis of the records taken in 1930.

Of the suckers planted out in November, 1929, ten arose from roots which were discoloured by *Stereum purpureum* at the points whence the suckers sprang, while in eleven more it could not be determined whether the fungus just did or just did not reach the suckers. None of the ten from definitely discoloured portions of roots recovered from the disease in 1930 ; those which were not dead still showed the silvering symptoms. From some of those which died *Stereum purpureum* was isolated. Two of these suckers appeared to be free from silvering in the early summer of 1930 but both were weak and bore only a few small leaves. They died during the summer and *Stereum purpureum* was isolated from one of them.

Of the eleven suckers attached to portions of roots where the presence of the fungus was uncertain only one has recovered. The recovery from the disease of suckers which arise from the discoloured parts of roots invaded by *Stereum purpureum* appears, therefore, to be exceptional, at any rate in the year after separation from the parent tree. Further recoveries may occur in subsequent years but, since the majority of these suckers contain within them the living fungus, it is unlikely that the proportion of such recoveries will be high.

Of forty-eight suckers which were taken from apparently healthy portions of root forty-three were silvered when attached to the trees. The majority of these became healthy in 1930, but the proportion which still showed silvering of the foliage was high—seventeen out of forty-three, or nearly 40 per cent. It is probable that most, if not all, of these will become healthy in time, since no sucker in this group appears to contain the fungus *Stereum purpureum*.

Group II. Suckers from Earith (Hunts).

Thirty silvered " Common Plum " suckers from an orchard at Earith were received in January, 1929. None of the ten examined in the laboratory had been invaded by *Stereum purpureum*. Of the twenty which were planted out, six died by the summer of 1929, but the death of all seemed to be due to root-failure and was certainly not due to Silver-leaf Disease. Eleven of the remaining fourteen became healthy in 1929, but the other three were still silvered ; in 1930 the condition of these suckers was the same as in 1929.

Group III. Suckers from Kent.

A large number of suckers from Kent were received in October, 1928. They were divided into two sections as follows :

(1) Suckers which had actually shown silvered foliage in 1928.
(2) Suckers which were alleged to have come from trees which died of Silver-leaf Disease.

Twenty-three of the suckers in Section (1) were cut up on arrival. None had been invaded by *Stereum purpureum*. Seventy-two others were planted out at

Melbourn. By the following July only thirty-two remained alive. Of the forty which had died five were examined in detail in the laboratory and a number of others were inspected more roughly in the field. The great majority appeared to have died from an inadequate root-system, but from one of the five examined in the laboratory *Stereum purpureum* was isolated. Two of the thirty-two suckers which were living in July, 1929, were slightly silvered, the remainder healthy. By September, 1929, twenty more suckers, all of which had been healthy in July, had died. These, together with those which had died earlier and which had not been examined were allowed to remain *in situ* in order to see whether any of them would develop fructifications of *Stereum purpureum* during the ensuing winter. No fructifications of the fungus appeared, and this confirms the view that the great majority had died not from Silver-leaf Disease but from an insufficient root-system. In June, 1930, only eight suckers remained alive. One of the two suckers silvered in 1929 had died while the other had become healthy, but one sucker which had been healthy in 1929 was slightly silvered in 1930.

In Section (2) there were 193 suckers, fifty of which were examined on arrival and found to be free from *Stereum purpureum*. The other 143 were planted at Melbourn. As in the suckers of Section (1), there were many deaths due to the failure to form roots, ninety-five of the suckers being dead by the end of the summer of 1929. In June, 1930, only forty-one remained alive. There was no evidence that any of the suckers of this section died from Silver-leaf Disease and all those which were living were free from silvering both in 1929 and in 1930.

Group IV. Suckers from Kent.

Another large batch of "Common Plum" suckers was received from Kent in January, 1928. This was sent divided into two sections, as follows :—

(1) Suckers which had actually shown silvered leaves, or which were attached to the same roots as those which had done so.

(2) Suckers believed to have come from the same trees but which were either leafless or had green leaves.

Eight of the suckers in Section (1) were examined on arrival and found to be free from *Stereum purpureum*, while twenty-five others were planted out at the University Farm. By the summer of 1928 eighteen of these had become healthy but the remaining seven still showed silvering. Of these seven, six were still silvered in 1929 while five of the eighteen which had become healthy in 1928 were silvered again in 1929. All the suckers were healthy in 1930. Two suckers which had retained their silvering symptoms for three successive years were examined in detail. One of them was cut up at the end of the second season after planting out, the other after the third season, during which it had become healthy. Neither showed any signs of *Stereum purpureum* although both contained small discoloured areas from which other fungi were isolated.

None of the sixty-nine suckers in Section (2) were examined in the laboratory. All were planted out at the University Farm and all were healthy in 1928. In 1929, however, five of them showed traces of silvering, but in 1930 they were all healthy again.

Group V. Suckers from Kent.

Forty suckers from the same source as those in Group IV, Section (1), were sent to a commercial nursery in Surrey in January, 1928, and were planted there. By July, 1928, three of them had died but the rest had become healthy. The three dead suckers were examined; death was apparently due to lack of roots. The thirty-seven healthy suckers were budded with various plum varieties in July, 1928, and their subsequent history is described later.

Group VI. Stocks in Kent.

The following silvered stocks were planted out at the Research Station, East Malling, in the winter of 1927-28:

36 Brussels,
21 Pershore, from the East Malling stool-beds,
2 St. Julien seedlings.

In 1928 all of these had become healthy except one of the Pershores, while in 1929 the same Pershore and one of the Brussels stocks showed traces of silvering. In 1930 all the stocks were healthy.

Several other smaller groups of silvered plum suckers were obtained from various sources. These suckers were either examined in the laboratory or planted out and kept under observation. The results obtained with these smaller groups closely resemble those described above.

The results of these investigations on silvered plum suckers can be summarized as follows:

(1) When a silvered tree, or one which has almost died from the disease, gives rise to silvered suckers the majority of these spring from clean roots and do not contain *Stereum purpureum*.

(2) The chances of recovery of silvered suckers which spring from discoloured parts of a root invaded by *Stereum purpureum* are very small indeed.

(3) Most silvered suckers which arise from clean roots become healthy in the year following their separation from the parent tree, but

(4) A fairly high proportion of such silvered suckers may retain the silvering symptoms for one season after separation from the parent tree, while a smaller

proportion may retain the symptoms for two seasons. Such suckers do not contain the mycelium of *Stereum purpureum*. None of the suckers under observation have shown silvering for three successive seasons after planting out, but it is possible that under other conditions they might have done so. In general, these suckers become healthy within a few years.

(5) Silvered suckers which have become healthy in the first season after planting out occasionally show silvering symptoms again in the second season.

It is difficult to account satisfactorily for the retention of silvering symptoms in those suckers which do not contain the living fungus. It is conceivable that buds laid down while the sucker is still attached to the silvered parent tree, and which therefore are under the influence of the toxic substance which causes silvering, might give rise to silvered leaves after detachment from the tree. But it seems very unlikely that any such mechanism could account for the retention of the silvering until the second season after planting out ; still less could it explain the reappearance of silvering after a season's absence as seen in some of the suckers in Groups IV and VI. It should be mentioned that the proportion of silvering seen in these suckers in the first two seasons after planting out is far too high to be accounted for by any spontaneous re-infection of the trees from without. Control batches of healthy suckers have been planted out at Melbourn and at the University Farm at Cambridge ; these controls comprise about 150 suckers and none of them has shown any signs of silvering. When silvering reappears, however, in detached suckers after a season's absence it is always slight ; possibly it is not really due to the influence of *Stereum purpureum*, but is comparable rather with the silvering sometimes seen in plum seedlings, in which the living fungus is certainly not present.

As regards the use of silvered suckers as stocks for nursery work, the danger does not appear to be very great. It lies in the possibility of such suckers containing the living mycelium of *Stereum purpureum*, but this is comparatively infrequent. The use of silvered suckers in propagation should, however, never be countenanced.

B. EXAMINATION OF WORKED STOCKS (YOUNG NURSERY TREES).

In "Silver-leaf Disease, IV," (1) it was stated that the disease in young nursery trees is frequently due to infection through wounds made in propagation. Since 1927 much more work on this matter has been done and the results confirm fully the conclusions arrived at in the earlier paper.

Most of this work has been carried out at Cambridge on worked trees received from commercial nurseries, but in addition a number of the stocks described in Part A were worked in various ways in 1928 and 1929. The results obtained with these trees will be considered first.

It was mentioned above that forty silvered suckers from Kent (Group V) were sent to a commercial nursery in Surrey in January, 1928, and that thirty-seven of these were healthy in the following summer. These suckers were budded in July, 1928, with Czar, Giant Prune, Early Rivers, Belle de Louvain, Victoria and Purple Pershore. Some of these buds failed to establish themselves, notably Czar and Early Rivers. The failures were re-budded with Victoria in July, 1929. All these trees have remained quite free from Silver-leaf Disease.

As previously described, the great majority of the stocks at East Malling (Group VI) had become healthy by the summer of 1928. About half of these healthy stocks were grafted with Victoria in March, 1929. There were some failures, but the trees have shown no signs of Silver-leaf Disease.

In the winter of 1927-28, twelve maiden peaches worked on Brompton were received from a commercial nursery. These had been budded before 1927 and the scions were fairly well developed on arrival. All had been silvered in 1927. The varieties were Peregrine (3), Duchess of Cornwall (2), Bellegarde (1), Sea Eagle (3) and Royal George (3). In 1928 five of the twelve trees died and fructifications of *Stereum purpureum* appeared on all of them. Of the remaining seven, two were heavily, three moderately, and one slightly, silvered, while one had become healthy. In 1929 the two heavily silvered trees died and the three moderately silvered trees were all heavily silvered, but the tree which was slightly silvered in 1928 had become healthy. In 1930 the survivors showed some improvement, one of the trees heavily silvered in 1929 being only moderately silvered while another had become healthy. The top of this tree had died, but there was abundant and vigorous healthy growth from the lower part. The condition of this group of trees three years after planting was, therefore :—

7 killed by Silver-leaf Disease (5 dead in 1928),
1 heavily silvered,
1 moderately silvered,
3 healthy.

At the same time twenty-five young plum trees of various kinds were received from the same nursery. These had also been worked before 1927 and the scions were fairly well developed. Twenty of these trees were known to have been silvered in 1927 ; nothing was known about the condition of the other five. In 1928 nine of the trees died from the disease, eleven were silvered and five were healthy. In 1929 two more of the silvered trees had become healthy, while in 1930 much improvement occurred and only two trees showed any silvering. After three years the condition of these trees (the majority of which were Early Rivers and Belle de Louvain) was as follows :—

9 killed by Silver-leaf Disease (all dead in 1928),
2 slightly silvered,
14 healthy.

In December, 1927, seventy-two young plum trees of various varieties were received from another nursery firm. Of these, forty-seven had been silvered and twenty-five healthy in 1927. All the stocks had been budded in 1926. Seventeen of the silvered trees were examined in the laboratory and thirty were planted out. All the healthy trees were planted. Of the seventeen trees which were examined, sixteen showed discoloration of the wood of the stock, due to infection by *Stereum purpureum*. In nine out of thirteen attempts the fungus was isolated from the discoloured region. In some of the stocks the mycelium had penetrated as far as the roots. The wood of the scion was usually healthy, but in one case the fungus had entered it. In the course of budding, three wounds, through which the spores of *Stereum purpureum* may gain entry, are regularly made in the stock. These are:

(1) the wound at the point of budding,
(2) the wound at the top of the snag of the stock to which the young scion is often tied,
(3) the wound made when this snag is removed.

All the silvered stocks examined seemed to have become infected through one or other of the last two wounds. The infection never appeared to proceed from the point of budding, where the wound is, of course, comparatively small. Twenty of the thirty silvered trees which were planted out in December, 1927, were dead by September, 1928, and *Stereum purpureum* was fructifying on nineteen of them. Six of the remaining ten were silvered. In 1929 seven were silvered and three healthy, while in 1930 the number of silvered trees was only four. Three of these, moreover, showed only traces of silvering. After three years the condition of these trees was, therefore:

20 killed by Silver-leaf Disease (all dead in 1928),
4 slightly silvered,
6 healthy.

The twenty-five healthy trees which were planted out in December, 1927, have all remained free from the disease.

In all, sixty-seven silvered trees, budded one year previously, were planted out and, of these, thirty-four died from the disease within a year of planting. Thirty-one of the trees were still living in 1930 and twenty-three of these had become healthy. A good deal of this improvement took place in 1930, which was undoubtedly a year in which recovery from Silver-leaf Disease was particularly evident.

The investigations described above again indicate that the disease in budded stocks nearly always begins at one or other of the wounds made in cutting back the stem of the stock. It should be possible to prevent the infection of such stocks (and therefore the young nursery trees) by protecting these wounds, immediately they are made, with soft grafting wax.

No evidence that the disease exists in a " latent " form in young nursery trees has been found. All the trees received as healthy from the nurseries have remained so during the three years during which they have been under observation after replanting. It is probable that the young trees which became silvered in circumstances such as might give rise to the idea of " latency " were infected by *Stereum purpureum* entering through the comparatively numerous wounds made in the course of planting, pruning and cultural operations.

VIII. GENERAL REMARKS.

During the last ten years we have been impressed by the large amount of recovery from Silver-leaf Disease shown even by very susceptible varieties of plums such as Victoria and Czar, provided the trees are growing vigorously. Such recovery has been particularly noticeable in the year following a hot, dry summer, notably after 1921 and 1929. Under weather conditions of this kind the trees appear to be stimulated to form gum-barriers, with the result that the fungus makes no further progress and sooner or later dies. We have seen several plantations which at first sight appeared to be ruined by the disease, but which after a few years recovered to such an extent that silvering of the foliage was no longer conspicuous in them. It has already been pointed out that provided a tree does not die during the first few years of attack the chances of complete recovery are good. If these facts are borne in mind, Silver-leaf Disease should not be looked on as being such a serious menace to fruit growing as it has been in the past.

On the other hand, the most serious outbreaks of the disease have occurred after seasons of heavy cropping, especially when these have been followed by a particularly wet autumn. This is undoubtedly due to breakage of the branches and the liability of the wounds thus made to invasion by the fungus. Under such conditions the disease may assume almost epidemic proportions in the following year. It is at such times that special precautions should be taken : as soon as the fruit has been picked the broken branches should be cut off and the exposures covered immediately with white lead paint or soft grafting wax.

In previous papers of this series the desirability of thinning-out fruit trees during the early part of the summer rather than in the winter has been advocated in order to minimize the risk of infection by *Stereum purpureum* ; further experience has amply confirmed this. Even with ordinary pruning operations, e.g. in shaping young trees, the less cutting that is done in the winter, the better. It is admitted that if the branches of young trees are cut back before the leaves fall there will probably be some decrease in growth, but with the exuberant Victoria plum this will be no detriment. If, for instance, the branches of young trees of this variety are shortened at the end of August there will be a reasonable chance of the speedy formation of gum-barriers to occlude the wounds, thereby preventing attack by *Stereum purpureum.* It may be suggested that cutting at this time will lead to proliferation of the buds before the autumn closes, but in our experience this does not happen. In shaping young trees any necessary cutting should be done at regular intervals from the nursery stage onwards so that only one year old twigs are shortened or excised, for wounds in such young tissues are less liable to invasion by *Stereum purpureum* than are exposures of older wood.

IX. SUMMARY.

1. The results of further experiments with various wound-protectives are given. Soft Grafting Wax and home-made White Lead Paint have again proved most satisfactory in preventing invasion of wounds by *Stereum purpureum.*
2. Various derivatives of "selfed" Victoria plums have been tested for susceptibility to Silver-leaf Disease. Considerable differences in susceptibility were found to exist and two of the most resistant types have been selected for further trials.
3. Spore-inoculation experiments have been carried out to test the susceptibility to Silver-leaf Disease of Victoria plums worked on different stocks, but the experiments could not be continued for a sufficiently long period to give conclusive results. Any influence which the stock may have on the susceptibility of the cultivated variety is probably of an indirect nature.
4. The use of the term "gum-barrier" is extended to include certain reactions of the host below wounds uninvaded by parasitic fungi. The formation of such gum-barriers is described ; they usually develop within an inch of the wound and require at least two months for their completion.
5. The results of further experiments on the formation of gum-barriers in association with invasions by *Stereum purpureum* are described, and the mechanism of gum-barrier formation in these circumstances is discussed.
6. Further experiments on the manurial treatment of silvered plum trees are described ; in one of these some correlation exists between the vigour of the tree and the amount of recovery from the disease. No one manurial treatment for Silver-leaf Disease can be recommended, but anything which increases the vigour of growth of the trees is likely to assist their recovery. In our experience phosphatic and potassic measures have been most helpful in promoting recovery.

7. The incidence of Silver-leaf Disease in plum suckers, stocks and young nursery trees has been investigated. Silvered suckers, devoid of the mycelium of *Stereum purpureum*, sometimes retain the silvering symptoms until two years after separation from the parent tree. In numerous young worked trees affected by the disease the fungus had entered through one or other of the wounds made in cutting back the stem of the stock in the process of propagation. The bearing of these results on nursery practice is discussed.

8. Hot, dry summers promote recovery from the disease. Provided that a tree does not die during the first few years of attack its chances of recovery are good. The greatest danger of widespread infection occurs after heavy cropping leading to breakage of the branches, especially when followed by a wet autumn.

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OBSERVATIONS ON THE VARIETAL RESISTANCE OF THE APPLE TO SCAB (*Venturia inaequalis*, Aderh.) WITH SPECIAL REFERENCE TO ITS PHYSIOLOGICAL ASPECTS.

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INTRODUCTION.

Although one of the most general and widely distributed diseases of the apple and one which causes much damage every year, Apple Scab may be both localized and variable as regards the intensity of its attack. In certain seasons the disease may reach almost epidemic proportions, while in other years it may be much more restricted.

The metabolic activity of the parasite unquestionably is an important factor in determining the intensity of an attack, but is not the only one concerned.

The cultivated forms or "varieties" of the apple, although extremely closely related botanically, show considerable variation in their resistance to the disease. The fungus may infect leaves, fruit and young wood of the apple; these organs may vary in their liability to attack in different varieties, and the type of lesion caused by the parasite often has an appearance characteristic of the variety of the host. "Varietal resistance," however, is not altogether constant, certain varieties may be more resistant in one season than in another, in one country than in another, or under one set of soil conditions than under another. Such "varietal resistance" or "varietal susceptibility" must ultimately be referred to the physiological peculiarities of the host varieties, when under uniform conditions of fungal attack.

Any investigation into the determining causes of the intensity of attacks of the Apple Scab fungus, *Venturia inaequalis* (Aderh.) must therefore involve a knowledge of the life history and physiology of both parasite and host. The present contribution on this subject embodies the results of the author's researches during 1927-29 at the Long Ashton Research Station, where exceptional opportunities are afforded for the study of apple trees of culinary, dessert and cider varieties, growing under various conditions of cultivation. The chief aims of the research were as follows:

(1) to elucidate the environmental conditions under which the fungus is able to attack the host-plant, and the possible modification of these conditions associated with different varieties of the apple,

(2) to study the effect of the host on the parasite, both at the time of penetration and during the growth of the fungus beneath the cuticle.

The effects of different varieties of apple and those of the same variety under various conditions were all involved.

It was not to be expected that in the limited space of the two years which the author was able to devote to the investigation, while holding a Bathurst Research Studentship from Newnham College, Cambridge, these aims should be fully attained. It was, however, hoped that a survey of the individual problems involved in the manner indicated below would serve to clear the field for future investigators of this important economic disease and to bring into proper perspective the various factors concerned with varietal resistance in this particular case. Thus it cannot be claimed for the data recorded that they are sufficiently exhaustive to be conclusive for most of the specific points discussed : on the other hand they do appear to give a lead as to the directions in which the predominant factors affecting resistance may be found.

OUTLINE OF INVESTIGATION.

From the foregoing it follows that any comprehensive investigation of varietal resistance to the Scab fungus must include consideration of conditions affecting the parasite as well as the host. Hence in the present investigation three main aspects have been studied : (1) environmental conditions affecting the fungus, (2) penetration by the fungus and (3) conditions affecting the growth of the fungus beneath the cuticle.

Conditions suitable for the spores of the fungus to germinate on the surface of the host are an essential preliminary to infection. The requirements of the fungus were studied and the possibility of varietal differences in the surface of the plant being significant was considered.

Penetration by the fungus was not always found to follow after spore germination. Some variation in the " virulence " of the parasite was observed, as well as variation in the susceptibility of the host to penetration.

The growth of the fungus within the host plant must be dependant on suitability of the plant sap as an environment. Wiltshire (1915) made some interesting preliminary investigations of the effect of liquids expressed from apple leaves on the growth of Scab spores. These observations were extended with reference to differences in the nature of liquids derived from different varieties, and to peculiarities associated with special physiological conditions of the same variety. Parallel studies were made of the state of resistance or susceptibility of the host and the extent to which the two types of observation can be correlated is considered.

The work is described in two parts. Part I is an account of the conditions affecting germination and penetration by the fungus. Part II deals with experiments in which an attempt was made to correlate varietal resistance with the effects of expressed liquids upon spore germination.

A bibliographical summary of previous work on *Venturia inaequalis* will not be given, as full lists of references have been published by Wallace (1912), Keitt and Jones (1926), Wilson (1928) and Salmon and Ware (1924).

PART I. GERMINATION AND PENETRATION OF THE HOST BY THE FUNGUS.

A. SPORE GERMINATION.

Spore germination is considered in connection with (1) field conditions affecting the spore, particularly as regards desiccation, (2) the nature of the surface of the host and (3) the vitality of the parasite.

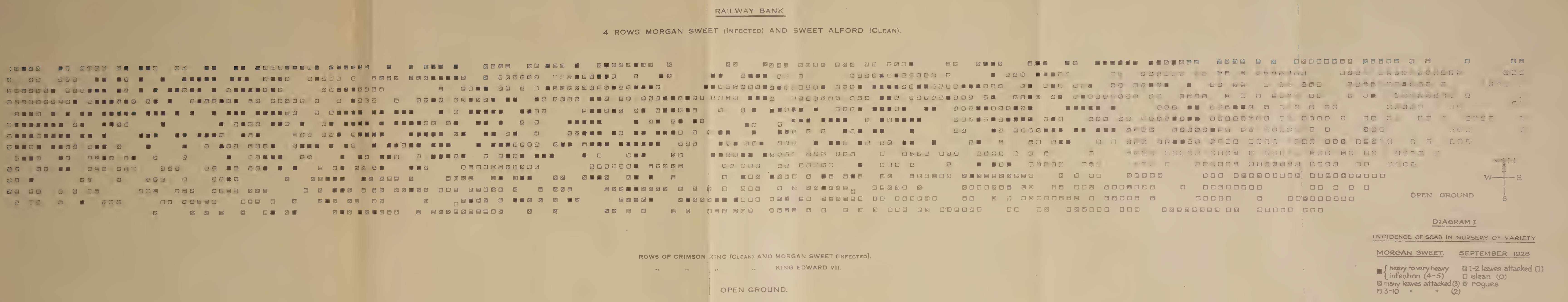
1. Field Conditions Affecting Germination.

Among the factors associated with spore germination in *Venturia inaequalis*, water relations appear to be of greatest importance. Other factors studied by Doran (1922) will not be considered here. The close association of Scab infection with wet weather is common knowledge, and humidity of the atmosphere, therefore, within a plantation may play an important part. A plantation in which one part is constantly more humid than another, where differences in the rate of the evaporation of water are marked, may show correlated variations in the distribution of Scab. A striking example was presented in the Kingscroft nursery at Long Ashton in September, 1928. The plantation consisted of trees of the varieties Sweet Alford, Morgan Sweet, Crimson King and King Edward VII, and, of these, infection was almost entirely confined to the variety Morgan Sweet. Fourteen rows of Morgan Sweet occurred in the centre of the nursery and inspection revealed that, while trees in the sheltered parts of the nursery were heavily attacked by Scab, those more exposed to sun and wind were relatively clean. Weather conditions had probably approached the minimum humidity requirements of the fungus; while sufficiently damp periods obtained in the sheltered parts, greater exposure reduced the length of the period of wetting below the minimum. (See Diagram I.)

Desiccation of Spores. Experiments were carried out in the laboratory and in the field in an attempt to ascertain whether inadequate wetting, followed by desiccation of the spores, merely delayed germination or whether the germinating power of the spores was permanently affected. It was observed that if spores were wetted for a short period and then dried, the power of germination

DIAGRAM I.

PLAN OF KINGSCROFT NURSERY SHOWING INCIDENCE OF SCAB INFECTION.


RAILWAY BANK
4 ROWS MORGAN SWEET (INFECTED) AND SWEET ALFORD (CLEAN).
NORTH
W
E
S
OPEN GROUND
ROWS OF CRIMSON KING (CLEAN) AND MORGAN SWEET (INFECTED).
" " " " KING EDWARD VII.
OPEN GROUND.
DIAGRAM I
INCIDENCE OF SCAB IN NURSERY OF VARIETY
MORGAN SWEET.
SEPTEMBER 1928
■ { heavy to very heavy infection (4-5)
▨ many leaves attacked (3)
□ 3-10 " " (2)
□ 1-2 leaves attacked (1)
□ clean (0)
☒ rogues

was considerably reduced. For drying out the liquid, desiccators containing sulphuric acid of known specific gravity were used, according to the method described by Stevens (1916). The desiccation is indicated by the use of the term "saturation deficit," i.e. the difference between the vapour pressure density and the maximum V.P.D. possible at the same temperature.

Drops containing a suspension of spores from pure culture in redistilled water were placed on coverslips. The slips were dried in the desiccators, a fresh drop of water was then added and the spores left to germinate for twenty-two hours. Control coverslips were placed in a damp atmosphere from the start. Groups of six coverslips were placed under each set of conditions and the results show the mean percentage germination in each group.

A study was made of

(a) the effect of varying the length of time of desiccation,
(b) the effect of repeated wetting and drying,
(c) the effect of the degree of saturation deficit,
(d) the influence of the surface on which the spores were lying.

Table I shows the effects of desiccation on the germination of the conidia.

TABLE I.

Effect of Desiccation on the Germination of Conidia of Venturia inaequalis.

		Treatment of Liquid.		
Desiccator conditions.		Dried for 4 hours. 29-2-28.	Dried for half hour.	Twice Dried and rewetted. 1-3-28.
Sp. Gr. of acid.	Sat. def. at 20 c.	Per cent. germination.	Per cent. germination.	Per cent. germination.
Water controls.		60.59	64.64	
1.12	1.3	25.59	32.44	
1.25	5.2	9.9	8.21	
1.30	7.3	16.28	24.14	
Water controls.			82.25	
1.12	1.3		41.94	25.64
1.25	5.2		29.2	17.41
1.30	7.3		23.82	19.48

These results emphasize the importance of the process of wetting and drying in causing loss of viability in contrast to the length of time of desiccation and degree of saturation deficit. In similar studies, Heald and Studhalter (1915) observed a loss in viability amounting to 86-94 per cent. by the ascospores of *Endothia* after washing in water. They thought that desiccation might remove a protective mucilaginous membrane and such a hypothesis might be applied to these results with *Venturia inaequalis*. The spores tend to become fastened to the coverslip during germination ; desiccated spores become even more firmly attached, suggesting that the mucilage might have been converted to an impermeable cement-like substance.

The effect of desiccation was modified by the nature of the surface on which the spore suspension was placed. For example, no loss of the power to germinate was observed on agar, indicating that the spores absorbed sufficient moisture from the substratum to prevent injury. Spores on the surface of storage fruits, a fairly impermeable substratum, suffered injury comparable to that on glass. For example, desiccation for fourteen hours on the skin of a Sweet Alford apple resulted in a reduction in germination of 51.53 per cent. and on glass of 49.82 per cent.

Injury to the spores also occurred on the leaf, although in the field exposure was much less injurious than in the desiccator. Drops of a spore suspension were placed on the leaf and the area marked by a circle of Indian ink. After the period of desiccation the spores were rewetted with distilled water and allowed to germinate in a damp chamber. After twenty-four hours the portions of the leaves, together with portions from undried leaves as controls, were decolourized with acetic alcohola nd stained with lacto-phenol and cotton blue. The germination of the spores on the surface could then be observed. Among the results obtained those shown in Table II are typical.

TABLE II.

Germination of Conidia after Desiccation and Exposure in the Field for Twenty-four hours, July 16th, 1929.

Substratum.	Germination of spores per cent.		
	Controls.	Spore exposed :	
		Desiccator.	In field.
Glass	96.29	10.08	—
Leaf : variety			
Sweet Alford ..	90.6	13.54	47.03
Crimson King ..	93.5	19.53	62.2
Morgan Sweet ..	95.35	29.33	64.65

Desiccation in the field entailed exposure to bright sunshine for about seven hours, followed by the comparative humidity of the night, and the effects of such exposure are observed to be less injurious than continuous exposure in the desiccator for twenty-four hours. Probably the spore is able to derive a certain amount of water from the living leaf in contrast to the gathered fruit. Some reduction in germination was, however, observed, which indicates that the spore is liable to injury by desiccation under certain field conditions.

2. GERMINATION UNDER FIELD CONDITIONS.

Field observations were made regarding the facility with which different organs of the tree were wetted and the rate at which they became dry, since any differences in these respects would clearly be significant in relation to varietal resistance.

(*a*) *Rate of Drying.*

Leaves. Observations were made in the field and greenhouse and with leaves in desiccators to find out if any differences in the rate of drying could be observed in different varieties. No special peculiarities were observed, but leaves with a glabrous, shining surface, such as those of the varieties Bramley's Seedling and Crimson King, dry relatively quickly. The leaves of these varieties, which are resistant, develop the glabrous condition of the upper surface earlier than some other varieties, such as Morgan Sweet and Worcester Pearmain (susceptible). Young leaves only are susceptible to infection ; hence the early development of a surface which dries readily is likely to be a factor associated with their relative resistance.

Fruits. The fruits of Sweet Alford have a waxy surface, from which water easily runs off, almost from the first. In this variety lesions are common either round the eye ("calyx infectiors") or on the lower surface, where drops of water have collected. In some other varieties, such as Cap of Liberty, the rate of drying of the surface as a whole is retarded by the presence of epidermal hairs, and it is significant that extensive lesions over the whole surface are commonly observed in these fruits.

(*b*) *Adherence of Water.*

When unfolding, most apple leaves exhibit a thick weft of hairs on the dorsal (lower) surface, which effectively hinders the penetration of water and prevents the germinating spores from coming in close contact with the epidermis. As Butler (1918) has observed, these hairs may well account for the comparative rarity of dorsal infections in Britain. Moreover, in a Siberian crab seedling raised at Long Ashton dorsal infections were as abundant as ventral infections, and here the dorsal surface became quite glabrous at an early stage in development.

Some young leaves are also protected by hairs on the ventral (upper) surface, and when these hairs persist until the leaf has almost reached maturity, as in many young nursery trees, an effective barrier to penetration is presented. Experiments in spraying young shoots of nursery stock with water in May, 1929, did not, however, yield any striking results as to varietal differences in the number of leaves wetted.

Another type of hair, which tends to absorb and retain water, occurs on the ventral epidermis of some varieties, e.g. Hohenzollern, which is highly susceptible.

Hairs of these two types also occur on the twigs and fruits. The twigs of Hohenzollern are hairy and water is absorbed, but usually hairs on the twigs prevent water penetrating to the cuticle, as in Beauty of Bath, Bramley's Seedling, Blenheim Orange and Morgan Sweet. Twigs on nursery stock are much more thickly invested with hairs than on the same varieties of old orchard trees, and the relative frequency of Scab in the orchard is probably connected with this fact.

In their young stages the fruits of many varieties are hairy. Water retaining hairs were observed on those of the variety Cap of Liberty (susceptible). Wetting was, however, rendered difficult by the hairs on the young fruits of Worcester Pearmain and Bramley's Seedling. These hairs disappear as the young fruit swells, and the most favourable time for the retention of water is a stage intermediate between the period at which the hairs form a thick covering and that at which the fruit has become glabrous and waxy. It may be noted in this connection that among the trees undergoing various manurial treatments in 1929, the young fruits of Bramley's Seedling on trees deficient in nitrogen and calcium appeared to be less thoroughly invested with protective hairs.

In general, hairs appear to be of supplementary importance rather than a factor determining varietal resistance to Scab. For example, they are evidently insufficient protection for the young fruit of Worcester Pearmain, but where no internal resistance is offered to the parasite by the host, as in Cap of Liberty, hairs retaining water render the plant liable to extensive lesions.

3. VITALITY OF THE PARASITE.

The vitality of the parasite and the viability of its spores are important both as regards germination and penetration and infection of the host.

1. VIABILITY OF SPORES. The viability of spores, indicated by their germination in water was affected by the following factors:

(a) Substratum of spore-producing lesion.
(b) Age of lesion or culture.
(c) Desiccation of lesion.
(d) Prolonged sub-culturing.

(*a*) *Substratum of Spore-producing Lesion.*

Highest germination was obtained from young lesions just showing an olive-green colour on young leaves. Conidia from fruits gave poor germination, and those from apparently fresh lesions towards autumn were practically inviable.

Good germination (up to 96 per cent.) was obtained with spores from cultures of good vitality on malt agar and gelatine and from apple agar, when sporulation occurred on this medium. A few cultures made on pith in Uschinsky's and Claussen's synthetic media gave but few spores and those were of low viability.

A comparison of the germination of spores in water from lesions of approximately the same age on leaves of different varieties gave the following results :—

	%
Sweet Alford	90.52
Cap of Liberty	84.89
An unnamed Cider variety ..	95.21
Court Royal	82.70

Lesions on certain substrata, e.g. the fruit and some culture media, yield spores of low viability, but such characters do not extend to spores from similar lesions of different varieties of the host.

(*b*) *Age of Lesion or Culture.*

The best germination was observed with spores which had just reached maturity. Those from old lesions or cultures showed a marked decrease in viability.

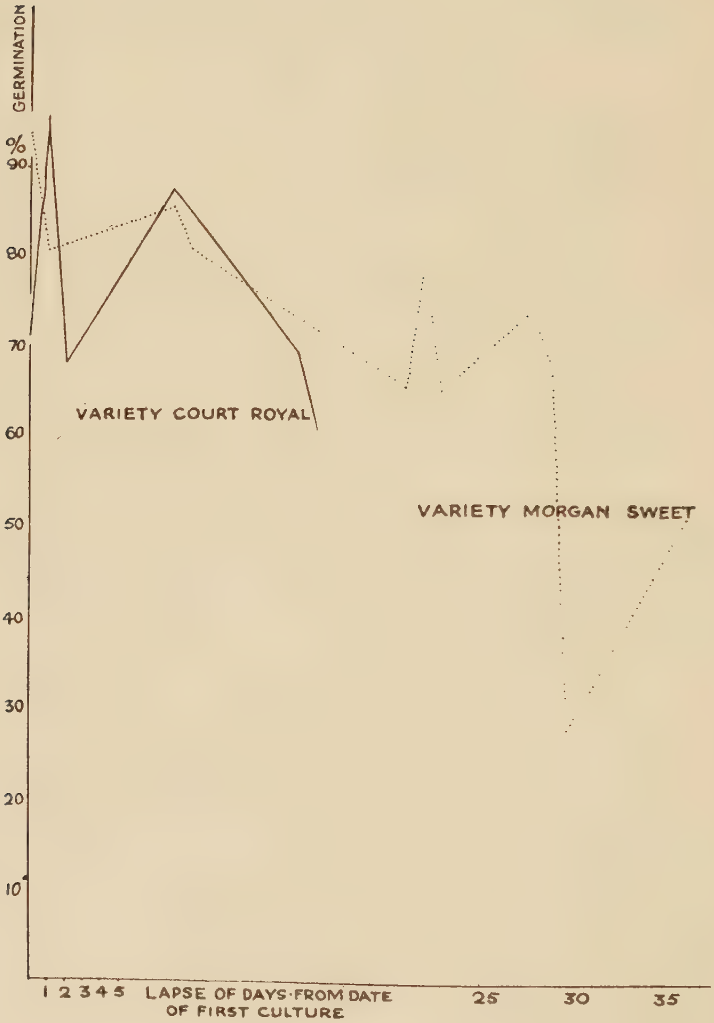
Variation in Lesions of Increasing Ages. Spores were used from leaves of Court Royal and Morgan Sweet. The nature of the outbreaks of Scab in each instance indicated that the infection of the tree occurred from a single attack ; thus all the lesions could be regarded as of about the same age.

TABLE III.

Germination of Conidia derived from Lesions on the Varieties Court Royal and Morgan Sweet at different times.

Court Royal.		Morgan Sweet.	
Date.	Per cent. germination.	Date.	Per cent. germination.
1928.		1928.	
July 25	71.0	September 19	94.01
,, 26	96.4	,, 20	80.64
,, 27	68.43	,, 27	85.54
August 2	87.59	,, 28	80.93
,, 9	69.04	October 10	65.06
,, 10	60.55	,, 11	78.13
		,, 12	64.74
		,, 16	73.64
		,, 17	68.12
		,, 18	26.68
		,, 25	52.97

These results are shown in graphical form in Graph I.



Graph I.

Relations between scab lesions of increasing age and the germination of their conidia.

Several reasons might be suggested to account for this decrease in viability. An increasing number of old spores which have lost their vitality through desiccation and other causes may become entangled with the conidiophores. Contaminations, especially yeasts, are common in old lesions and might affect viability of the conidia. Desiccation of the lesion may be of influence, although areas well washed by rain produce spores of low viability, indicating that the condition of the substratum or stroma is of more importance.

Germination of Spores from Culture. A similar decrease in viability is shown by spores derived from pure cultures.

Some typical results are shown in the accompanying table.

TABLE IV.

Germination of Spores from Cultures of Different Ages.

January 4th, 1928.		January 19th, 1928.	
Age of culture in days.	Per cent. germination.	Age in days.	Per cent. germination.
106	1.96		
96	5.20		
90	5.56		
69	7.55		
61	22.44		
55	12.42		
46	35.91		
41	4.48		
28	28.74		
26	58.78		
		19	30.03
		14	44.72
7	43.04	9	71.56

In the strain to which the figures used in these tests refer the maximum germination obtained about nine days after sub-culturing; in other strains of different origin the spores appeared to mature at different rates. For example, in February, 1929, a strain from Siberian crab gave best germination twelve days after sub-culturing and a strain from Morgan Sweet in not less than sixteen days. The crab strain had, however, been in culture for three months longer than that from Morgan Sweet.

(*c*) *Desiccation.*

Desiccation appears to be an important factor in hastening this loss of viability by spores. Portions of a malt agar culture kept in desiccators for four days gave spores of reduced viability, even though the agar was not completely dried out. Germination in the controls varied from 64.99 to 74.01 per cent. and in spores from cultures kept in the desiccators from 30.2 to 63.89 per cent., with a mean of 47.28 per cent.

A small loss in viability was observed in spores from a leaf placed in a desiccator for twenty-four hours. Here the germination fell from 95.76 per cent. to 72.93 per cent. On the whole, the figures suggest that during a spell of hot, dry weather some loss in viability of spores on the lesions would occur.

(*d*) *Prolonged Sub-culturing.*

Marked effects on spore viability are observed after prolonged sub-culturing.

(i) After some months the strain forms fewer conidia, later producing mycelium only. Similar phenomena were observed by Dastur (1919) in *Glomerella cingulata* and by Nattrass (1928) in *Physalospora miyabeana*. A strain isolated from Worcester Pearmain ceased to form abundant conidia during December of 1927, but show partial and temporary recovery of this faculty on apple agar, which usually favours mycelial development. The strain then became almost entirely mycelial until March, 1929, when a few conidia began to be formed once more. A strain isolated in June, 1928, produced good crops of conidia until April, 1929, when it began to deteriorate. An isolation from a perithecium-forming strain in September, 1928, yielded abundant conidia throughout the winter.

An observation of Aderhold (1896) is interesting in this connection. He found that isolations made in spring and early summer produced many conidia, and sub-cultures in the autumn, mycelium ; isolations made in the autumn also formed mycelium. He thought that the first generation from ascospores forms a greater proportion of conidia than the mycelial, perithecial-forming autumn generations. Isolations made by the present writer were far fewer than those made by Aderhold, but did not exhibit the same phenomena. It should also be noted that in *Glomerella*, Dastur observed a transition from the production of perithecia to conidia and, lastly, mycelium only in his cultures.

(ii) Another effect of prolonged sub-culturing is the appearance of spores very irregular in shape and somewhat resembling the *Cladosporium* type of spore or even irregular break-up of portions of mycelium. Germination is also abnormal, and may not be by septation and germ tube formation according to the usual plan, but by swelling and extrusion of the spore at one end to form a hypha. (See Figs. 1 and 2, Plate I.)

B. PENETRATION BY THE FUNGUS.

The germinating spore forms an appressorium either immediately or at the end of a hypha and from this an " infection thread " passes through the cuticle. It was soon found that the vitality or " virulence " of the parasite must be considered in this connection as well as in relation to spore germination. Also the resistance or susceptibility of the host plant must be taken into account.

PLATE I.

IRREGULAR SPORES FORMED AFTER PROLONGED SUB-CULTURING Sept: 1928 to April 1929

Fig: 1

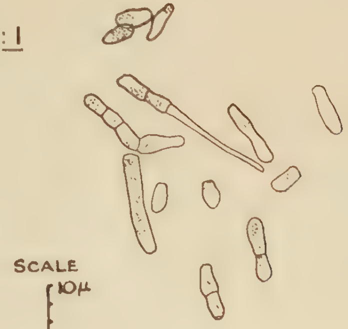
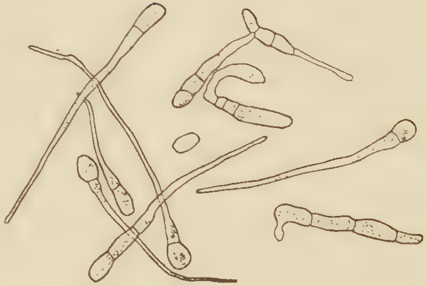


Fig: 2 24 HOURS GERMINATION



SEE Figs: 3-5 FOR COMPARISON WITH NORMAL SPORE FORMS

Fig: 3 FRESHLY DETACHED CONIDIA FROM A TWIG LESION

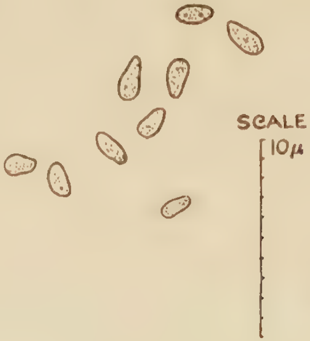


Fig: 4 AFTER 22 HOURS GERMINATION IN WATER

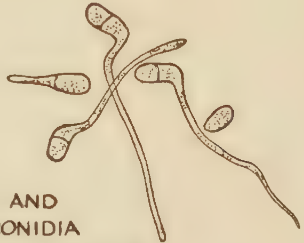
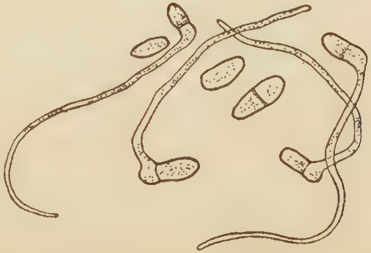


Fig: 5 GERMINATING AND UNGERMINATING CONIDIA FROM A MALT AGAR CULTURE (NORMAL TYPE)



1. VIRULENCE OF THE PARASITE.

(*a*) Some evidence has accumulated which suggests that different strains of the fungus vary in their power of parasitic attack. Aderhold made inoculations with conidia from different sources with the following results :—

Ascospores		33	per	cent	infection
Conidia	(leaf)	22	,,	,,	,,
,,	(fruit)	8	,,	,,	,,
,,	(culture)	poor			,,

Unsuccessful inoculations with conidia from cultures in the spring of 1928 and 1929 support this evidence of reduced virulence after culturing. Microscopic surface view preparations showed that development had proceeded as far as appressorium formation, but no penetration followed. Considered in connection with the work of Brown (1926) on penetration, this may suggest that in culture a gradual decrease in osmotic pressure occurs in the hyphæ, so that insufficient pressure is exerted to pierce the cuticle. Poor infections from fruit conidia are, however, to be considered in the light of inferior germination.

(*b*) A high percentage of infection was obtained with ascospores in the spring of 1929. Seven trees of Worcester Pearmain, undergoing different nutritional treatments, were inoculated with both ascospores and conidia from a subculture of an isolation made in the previous September from Morgan Sweet leaves. Portions of leaves bearing numerous perithecia were shaken up with water in a test tube. After an hour or two many ascospores had been liberated and the liquid containing them was used as an inoculum. The suspension was brushed on the leaves and the whole twig enclosed in a lamp shade, plugged at either end with damp cotton wool. After twenty-four hours the shades were removed, actual water being no longer essential for the fungus to develop, but in very dry weather a light watering was found to encourage the growth of the parasite in the leaf. The shades were supported by fine rings attached to stakes and covered with black paper to prevent scorching by the sun.

A suspension of conidia from a malt agar culture was similarly used as an inoculum.

Infection was obtained on all the shoots of Worcester Pearmain inoculated with ascospores, but with conidia from culture only two trees were infected—that obtaining full nutrients (A) and that deficient in potash (D).

Similar inoculations on trees of the variety Bramley's Seedling resulted in the infection of five trees with ascospores, the exceptions being trees deficient in nitrogen and those receiving rain water only. No infection was obtained with conidia from culture.

(*c*) The strain of the fungus used in the foregoing tests, known as N, was isolated in September, 1928, and appeared to be one of abnormal virulence. The Morgan Sweet trees in the Kingscroft nursery on which it occurred were very heavily attacked—some defoliation being caused. Perithecia, hitherto not commonly observed at Long Ashton, were abundant on the fallen leaves; and primary infections on the lower surface of the leaves were common in the spring.

The Morgan Sweet trees were again heavily attacked in 1929 and experiments were conducted in which infection by conidia from this source was compared with other "strains." In June, 1929, trees in the nutritional trials were inoculated with these conidia (strain N) and also with conidia from the leaves of Court Royal (strain R). The results are shown below; an x denoting infection and o a negative result.

			Strain N.	Strain R.
Bramley	A	(full nutrients)	x	o
	B	(lacking nitrogen)	o	o
	C	,, potash	x	o
	E	,, phosphates	x	o
	F	,, calcium	x	o
	G	,, magnesium	x	o
	H	,, rain water only	o	o
Worcester	A		x	o
	C		o	x (1 small lesion)
	D		x	x ditto
	E		x	o
	F		x	x
	G		x	o
	H		o	o

These results supported the view that the strain prevalent in the Kingscroft nursery was one of special virulence.

(*d*) The possibility of the existence of biological strains of the fungus, capable of infecting certain varieties of the host plant only, was also considered. Owing to their superior germinating ability, conidia from leaf infections rather than from cultures in artificial media were employed and the following "strains" were used:

N. conidia from leaves of Morgan Sweet in Kingscroft nursery.
A. ,, ,, ,, ,, Sweet Alford in Cider Orchard.
B. ,, ,, ,, ,, Bramley's Seedling.

The following results (Table V) were obtained :

TABLE V.

Date of inoculation.	" Strain " of fungus.	Variety of host.				
		Sweet Alford (Orchard).	Sweet Alford Nursery.	Morgan Sweet.	Crimson King.	Bramley's Seedling.
4–6–29	A	x (extensive)	x (extensive)		x (restricted)	o
	N	o	x (restricted)		x	x (extensive)
2–7–29	A	x (extensive)	x	o	o	x (restricted)
	B	x	x	x		x (extensive)
	N	o	x	x	x	x
18–7–29	A	(destroyed)	x (extensive)	x		x (v. restricted)
	B	(destroyed)	x	x		x
	N	x (restricted)	o			x (v. restricted)

These results suggest that possibly two groups of strains might be detected. The strains N and B appear to infect varieties Morgan Sweet and Bramley's Seedling most readily and the strain A the variety Sweet Alford, both in the cider orchard and in the nursery. The extensive infection of leaves in the nursery with this strain is notable, since the Sweet Alford trees there were almost entirely free from scab, although surrounded by heavily infected trees of Morgan Sweet. The strain R (from Court Royal), mentioned previously, may also belong to the A group, prevalent in the same orchard; as has been observed, no infection of Bramley's Seedling was obtained with it.

Of late years the variety Bramley's Seedling has become increasingly infected by scab, although for some time after it was first raised it was markedly resistant. Although still fairly resistant at Long Ashton, it has been badly attacked at East Malling during the last three or four years. In Northern Ireland the variety has been very susceptible for some time.

Such a loss of resistance may be due to changes in the physiology of the host plant, possibly associated with prolonged cultivation of a variety, particularly by the vegetative methods in current use. On the other hand, new forms of the fungus may have arisen, giving rise to " strains " of increased virulence or specially capable of infecting certain varieties formerly resistant to the disease. Wallace (1913) thought that some such theory might account for the increased susceptibility of the variety Baldwin in New York. In 1892 it was considered to be fairly resistant, but in 1910 a normal tree yielded 98 per cent. of scabby fruit.

The general aspect of the epidemic observed in the Kingscroft Nursery in September, 1928, rather supported the hypothesis that a distinct strain of the fungus was present. The lesions were mainly of the diffused type shown by Wilson (1928) to be associated with perithecial development, and perithecia were formed abundantly on the fallen leaves. In pure culture, conidia were formed more irregular in shape and developed more in knots than in other cultures. The time of isolation might, however, be important. This feature, associated with perithecia formation, tends to suggest that this strain may be biologically specialized.

2. SUSCEPTIBILITY OF THE HOST PLANT.

Keitt and Jones (1926) have made it clear that each organ of the plant liable to attack—leaf, twig and fruit—passes through a period of maximum susceptibility, which varies with each organ. This period must, therefore, coincide with field conditions favourable to spore germination, if maximum infection is to result.

(1) *The Leaf.* Young leaves only are attacked, but as they are usually formed throughout the season, especially on young trees, a period of damp weather is usually followed by infection in the plantation. Experiments were made of inoculating successive leaves on a twig ; in the majority of shoots, where five leaves showed the transition from extreme youth to maturity, only the youngest became infected.

Some evidence for varietal differences in the length of the susceptible period was obtained ; the varieties Morgan Sweet and the susceptible variety U, mentioned later, showed a longer period of susceptibility than the varieties Sweet Alford, Crimson King and Lane's Prince Albert.

In a series of pot-culture manurial deficiency trials in progress at Long Ashton some infection experiments were also made in this connection. The following results (Table VI) were obtained when such trials were made.

TABLE VI.

		Infection Date.			
		May 15th.	May 29th.	June 17th.	June 26th.
Bramley	A	x 2 leaves.	x 1 leaf.	x penult. leaf.	x 2 leaves.
	C	x 1 leaf.	x 1 leaf.	o	o
	D	o	x 2 leaves.	x 1 leaf.	x 2 leaves.
	E	o	x 2 leaves.	o	x 1 leaf.
	F	x 2 leaves.	x 2 leaves.	o	x 1 leaf.
	G	x 1 leaf.	x 2 leaves.	o	x 1 leaf.
	H	o	o	x 1 leaf.	o
Worcester	A	x 2 leaves.	x 2 leaves.	x 2 leaves.	x 1 leaf.
	C	x 1 leaf.	o	x 1 leaf.	o
	D	x 2 leaves.	x 2 leaves.	x 1 leaf.	x 2 leaves.
	E	o	x 2 leaves.	o	x 1 leaf.
	F	o	x 1 leaf.	x 1 leaf.	x 2 leaves.
	G	x 1 leaf.	x 1 leaf.	x 1 leaf.	x 1 leaf.
	H	x 1 leaf.	o	o	o

The manurial treatments in the series A—H were as follows :—

A. Complete nutrients
C. Nitrogen omitted
D. Potash ,,
E. Phosphate ,,
F. Calcium ,,
G. Magnesium ,,
H. Rain Water only.

It is noteworthy that, although these results show that an absence of nitrogen tends to retard maturation of the leaf in general, in no tree characterized by lack of nitrogen (the C and H series) was more than the youngest leaf infected. The actual length of the susceptible period, apart from the size of lesion formed, appeared to be rather longer on the trees receiving full nutrients and in those lacking potash and possibly calcium.

(2) *The Fruit.* Keitt and Jones and others have emphasized the importance of early infection, particularly through the calyx, for such infection causes greatest deformation and cracking of the fruit. Late infection, e.g. in August, causing superficial spotting, was seen in certain varieties, particularly Sweet Alford. Waters (1922) and Morse and Lewis (1911) brought forward evidence that Scab can spread to sound fruits in cool storage. Others (Wallace, 1911, and Morse, 1910) consider that actual infection occurs earlier but that the results do not become apparent until during the storage period. This is also the present writer's view ; the incubation period for some Worcester Pearmain apples inoculated in June was thirty days and lesions were not observed until after rain. Attempts were also made in December, 1927, to infect storage apples of the varieties Cap of Liberty, Allington Pippin, Sweet Alford, Frequin d'Audierre and Bramley's Seedling. Although the spores germinated well, no infection was obtained.

(3) *The Twig.* Twig infections were abundant in the autumn of 1927, but less so in 1928. July, 1927, was a wet month and July, 1928, very dry. Probably July, the time when twig lesions are first apparent, is the susceptible period and it is then that suitable weather conditions are necessary for this type of infection.

3. PENETRATIBILITY OF THE CUTICLE.

Wiltshire (1914) demonstrated the manner of penetration by the fungus by an infection hypha.

The cases he investigated suggested that little obstacle to penetration was afforded, even by the resistant host. Observations made in 1928 indicated, however, that penetration does not invariably occur.

Actual thickness of the cuticle has no effect on penetration by the parasite, which is able to pass through the thickest layers of cuticle on the fruit and petioles of susceptible varieties.

Some resistance to penetration was, however, observed in microscopical preparations of leaves of different ages, which indicated qualitative differences in leaf cuticles. In shoots of Lane's Prince Albert, Crimson King and King Edward VII the fungus was observed to have entered the youngest leaf inoculated (see Fig. 15, Plate III). In the older leaves a germ-tube and appressorium could be seen but no penetration had occurred (see Figs. 16-18, Plate III). The fungus entered five successive leaves on shoots of susceptible varieties inoculated as controls (a susceptible seedling known as Seedling A, and Morgan Sweet). (See Figs. 6-14, Plate II.) It made vigorous development in the first three leaves (Figs. 6-10, Plate II). The writer would suggest that the time during which certain varieties, of which these are examples, are susceptible to penetration and infection is longer than the period of susceptibility of varieties exemplified by Lane's Prince Albert. The weaker development observed in the two oldest leaves of the Seedling A (Figs. 11-14, Plate II) suggests that the age is being reached at which infection cannot be established.

In the resistant variety Grenadier, penetration occurred in the five leaves (see Figs. 19-25, Plate IV) but the mycelium appeared to be of weak development and stained lightly. This result is in accordance with results obtained by Wiltshire (1915), who observed that penetration, followed by disintegration of the mycelium, occurred in both old leaves of susceptible varieties and all leaves of resistant varieties.

The results mentioned above indicate, however, that in some varieties the impenetrable nature of the cuticle is an obstacle to the infecting hypha.

SUMMARY—PART I.

1. The importance of an adequate water supply for spore germination in *Venturia inaequalis* is emphasized. Desiccation, following wetting, is injurious to the conidia and the influence of different substrata on the effects of desiccation is shown.
2. Wetting of the host organ is modified by some varietal peculiarities in the apple. Protective hairs are present in some young leaves, fruits and twigs, and in a few varieties water-retaining hairs are present. In general, varietal characters of this kind are evidently of supplementary importance only in relation to resistance and susceptibility to Scab.
3. Variation in the vitality of the parasite has been observed, affecting both spore germination and the infecting power of the fungus. Conidia showed a reduction in germination when derived either from certain unfavourable

PLATE II.

FIGURES ILLUSTRATING THE RESULTS OF INOCULATIONS

SUCCESSIVE LEAVES ON YOUNG SUMMER SHOOTS INOCULATED, YOUNGEST LEAF JUST UNFOLDING. INFECTION IS SHOWN AFTER 11 DAYS.
INOCULATION JUNE 22nd 1928, FIXATION (ACETIC ALCOHOL) JULY 3rd 1928

SECTIONS 10μ IN THICKNESS
STAINED COTTON BLUE & SAFRANIN

FIGURES TRACED
WITH CAMERA LUCIDA

SCALE THROUGHOUT

I. VARIETY: SUSCEPTIBLE SEEDLING A.
NOTE PENETRATION IN FIVE SUCCESSIVE LEAVES AND VIGOROUS GROWTH IN LEAVES 1 TO 3

Fig: 6
Leaf 1 (youngest)

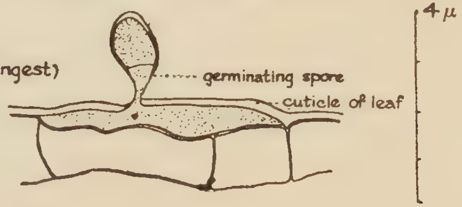


Fig: 7 Leaf 1

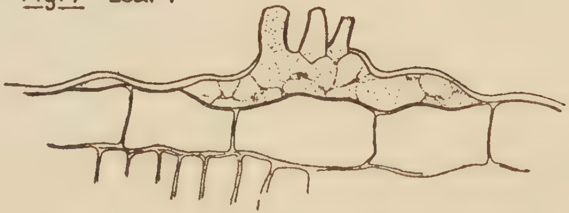


Fig: 8 Leaf 2

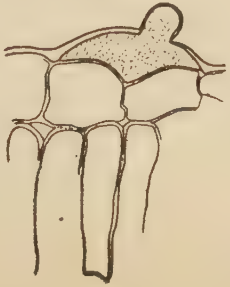


Fig: 9 Leaf 2

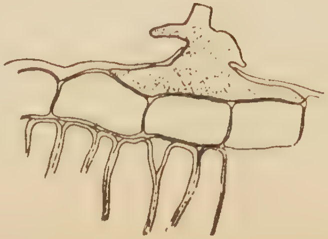


Fig: 10
Leaf 3

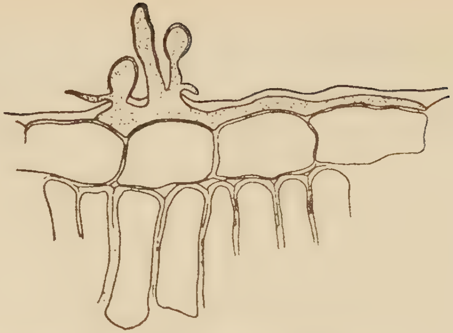


Fig: 11 Leaf 4

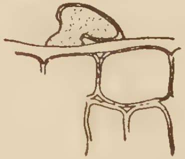


Fig: 12 Leaf 4

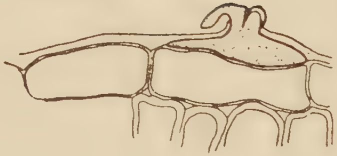


Fig: 13
Leaf 5

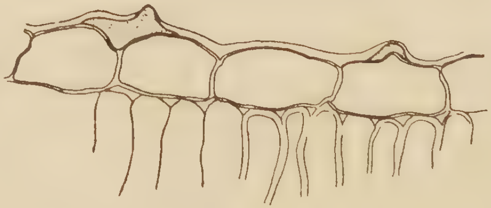


Fig: 14
Leaf 5

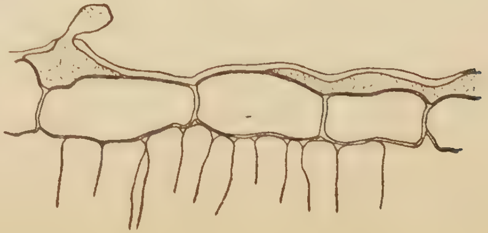


PLATE III.

II. VARIETY: LANE'S PRINCE ALBERT

NOTE PENETRATION IN YOUNGEST LEAF ONLY

Fig: 15 Leaf 1

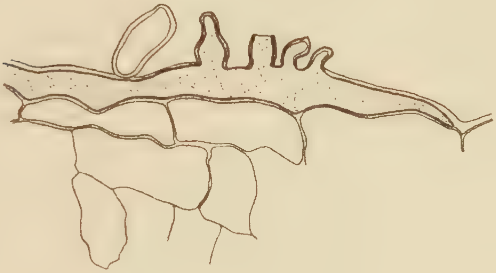


Fig: 16 Leaf 2

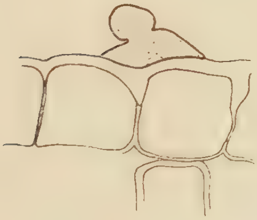


Fig: 17 Leaf 3

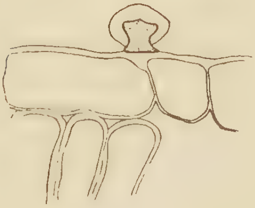
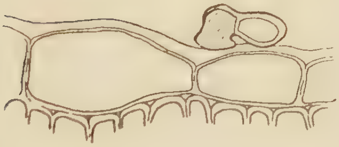


Fig: 18 Leaf 4



Leaf 1. A young leaf. The ages increase in Leaves 2, 3 and 4.

PLATE IV.

III. VARIETY: GRENADIER

NOTE PENETRATION IN MAJORITY OF LEAVES BUT MYCELIUM NOT VIGOROUS

Fig: 19 Leaf 1

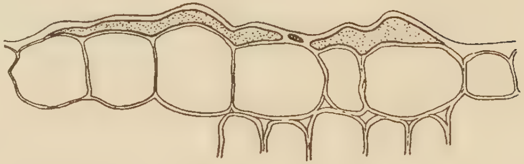


Fig: 20 Leaf 1

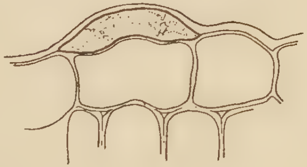


Fig: 21 Leaf 2

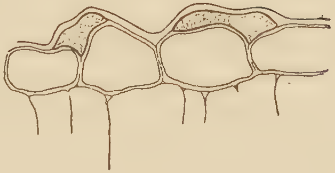


Fig: 22 Leaf 2

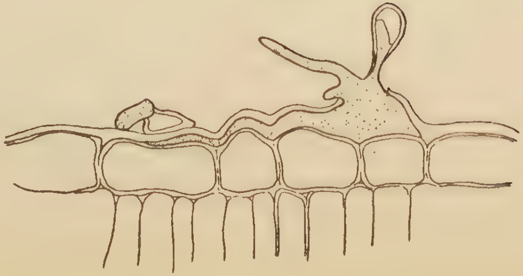


Fig: 23 . Leaf 3

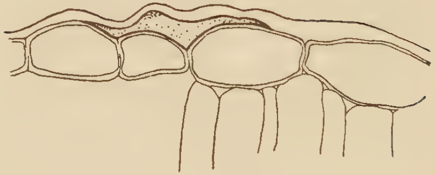


Fig: 24 Leaf 4

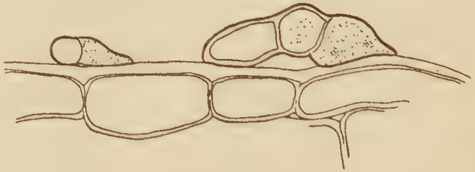
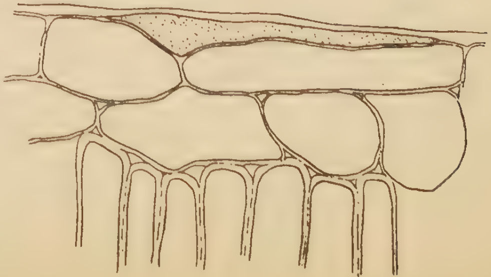


Fig. 25 Leaf 4



substrata, or a desiccated lesion. Conidia gave an optimum germination when just mature. Prolonged sub-culturing gave rise to several abnormalities.

Conidia from culture and certain strains had a low infecting power. One strain, known as N., appeared to be one of special virulence ; it was also a perithecia-forming strain.

4. Experimental work suggests that some biological specialization of strains of the fungus to certain groups of varieties exists.
5. The organs of the host plant pass through a period of maximum susceptibility ; the length of the period varies. It is significantly longer in certain susceptible varieties and in trees deficient in potash or, possibly, calcium.
6. Actual thickness of the cuticle presents no barrier to penetration, but in certain varieties an impenetrable quality appears to develop, so that the period of susceptibility is of shorter duration in these varieties.

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(*To be continued.*)

(*Received* 16/1/31.)

APPLE SCAB CONTROL IN THE BRISTOL PROVINCE: FIELD TRIALS, 1930.

By R. W. MARSH.

INTRODUCTORY.

In spraying trials against apple scab carried out in the Long Ashton plantations in the past three years, excellent control has consistently been obtained by spray applications terminating at or before the petal fall stage. For the 1930 season it was decided to test this method of control more widely by making field trials in commercial plantations in Somerset, Worcestershire and Herefordshire. During the late summer and autumn of 1929 four centres were chosen for the spraying trials of 1930: these centres will be referred to as Bridgwater, Yeovil, Hereford and Malvern. The chief motive in choosing the Bridgwater, Yeovil and Hereford orchards was that the crops at these centres were completely ruined by scab in 1929. The Malvern plantation was chosen in order to repeat a trial at this centre which had on a previous occasion been spoilt by capsid attack. The four centres chosen were alike in being primarily laid out for the production of dessert apples, but in other respects they were very diverse, including examples of grass orchards and plantations, of trees of varying ages, of orchards well-equipped for spraying and those poorly equipped. Because of this diversity, each centre will be described individually in a subsequent section.

SOURCE OF SCAB INFECTION IN THE SPRING.

The location of the spraying trials in commercial plantations made the provision of large blocks of control trees not feasible. In each centre, however, a small group of trees was left unsprayed, so that some indication of the normal intensity of scab attack during the year could be obtained. In addition to taking the usual field observations on the spread of the disease, the attempt was made to determine the date at which infective material of the scab fungus could first be detected in the air round the trees and the form in which it was present. Two possibilities existed—the infective material present in the spring might either be ascospores from over-wintered leaves or conidia from wood pustules. These two types of spore are easily distinguishable microscopically, but the conidia from the wood are the same as those produced later on the leaves and fruit. It followed that only those conidia present in the early stages of the unfolding of the leaf could be considered definitely as having come from the wood pustules, since at that period there would not have been time for conidia-bearing pustules to form on the young leaves. The attempts to record the number and type of spores present in the air were therefore confined (with one exception) to the period of approximately a month after bud-break.

The method of recording the spore content of the air was by exposing ordinary microscope slides (3 inches by 1 inch) of which both sides had been thinly coated with vaseline. These slides were suspended vertically in various directions from wooden clips attached to the branches of unsprayed trees at differing heights above the ground. The trees employed were those in the grass orchards at the Yeovil and Hereford centres, and in the plantation at Bridgwater. After several days' exposure the slides were brought in to the laboratory, where measured areas of both surfaces were examined at a magnification of approximately 400 diameters, and the number of scab conidia and ascospores noted. From several such readings, the average deposition of spores per square inch of surface for the period of exposure was obtained.

SPRAY PROGRAMMES.

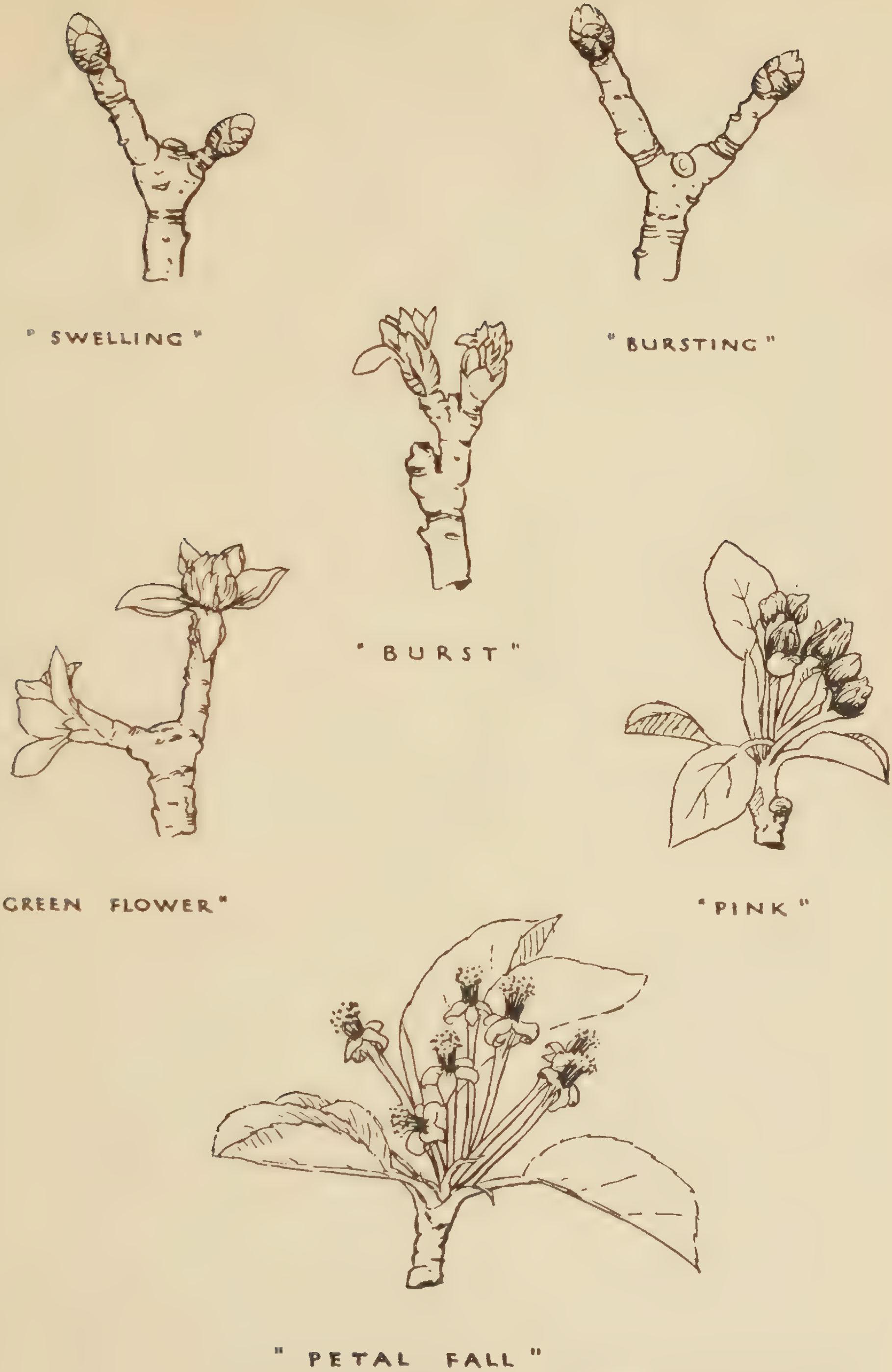
The general programme of spraying proposed for each trial was that which had proved so successful at Long Ashton, namely, two pre-blossom and one post-blossom sprays. This programme was carried out (except at Malvern) on all the trees of the badly-scabbing varieties (Worcester, Grieve, Cox) throughout the trials. However, on trees of Lane and Newton, which are less susceptible to scab but very sensitive to spray damage, pre-blossom applications only were given. The relation of the time of spraying to the stage of development of the blossom buds was recorded whenever possible. An explanation of the nomenclature used for the blossom bud stages is given by the diagrams in Fig. 1.

WEATHER.

As weather is an important factor in determining the intensity of scab attack, a brief summary of the 1930 conditions will be given. A very mild and wet winter was followed by a persistently dull, cool spring. From the middle of March until the end of April was a showery, cold and sunless period except for a short, mild spell at the end of March. The last few days of April and the first of May were fine, but afterwards dull, cool weather set in again with frequent showers but no heavy falls. At the end of May the temperature rose, and June was generally a fine and dry month with a short period of heavy rain in the third week. July was fair in the first fortnight, but later the cool, unsettled, showery conditions returned and continued until the end of August when there was a week of dry and very hot weather. September, after a fair beginning, was dull and rainy with gales and cold winds at the end of the month.

One result of the cold spring was that the opening of the buds in 1930 was very much later than normal, so that the blossoms entirely escaped frost damage. The percentage of setting was generally low, but the quantity of blossom was so great that, with a few exceptions, the varieties included in the trials carried a fair crop. Conditions for spraying were good in June, but earlier the number

BLOSSOM STAGES.



" SWELLING "

" BURSTING "

" BURST "

" GREEN FLOWER "

" PINK "

" PETAL FALL "

Drawings after N. J. Adamson.

FIG. 1.

of wet days severely limited the time available for spray treatments. Infection by the scab fungus was favoured by the weather in May but checked by the dry period in June and early July. After mid-July, however, the conditions for rapid spread of the fungus were highly favourable, and late attacks on fruit were general throughout the province.

Method of Recording Results.

Because of the amount of material involved, it was necessary, in recording the results of the trials, to make use of some system of sampling and to take samples which were a relatively small proportion of the total crop. In order to make the results as nearly comparable as possible, all the counts were carried out during the same week (the last week of August), and the samples of each variety were collected from several trees in different parts of the appropriate blocks. The samples were then carefully graded on the lines introduced by M. H. Moore* at East Malling to facilitate the comparison of results of spraying trials. Each apple on examination was placed in one of five categories dependent on the total area of scab infection, these five categories being as follows (see Fig. 2).

Category I	Entirely free from scab.
Category II	Scabbed area not exceeding a total of ¼ inch square.
Category III	Scabbed area exceeding a total of ¼ inch square but not exceeding a total of ½ inch square.
Category IV	Scabbed area exceeding a total of ½ inch square but not exceeding a total of 1 inch square.
Category V	Scabbed area exceeding a total of 1 inch square.

Details of Individual Spraying Trials.

For each centre the following details are listed in the order given :—

Description of orchard.
Previous scab attack.
Spraying equipment available.
Spray programme drawn up.
Dates of various stages in bud development.
Spore deposition.
First scab spray.
Second scab spray.
Third scab spray.
Spray damage.
Development of scab during summer.
Yield of clean and of scabbed fruit.

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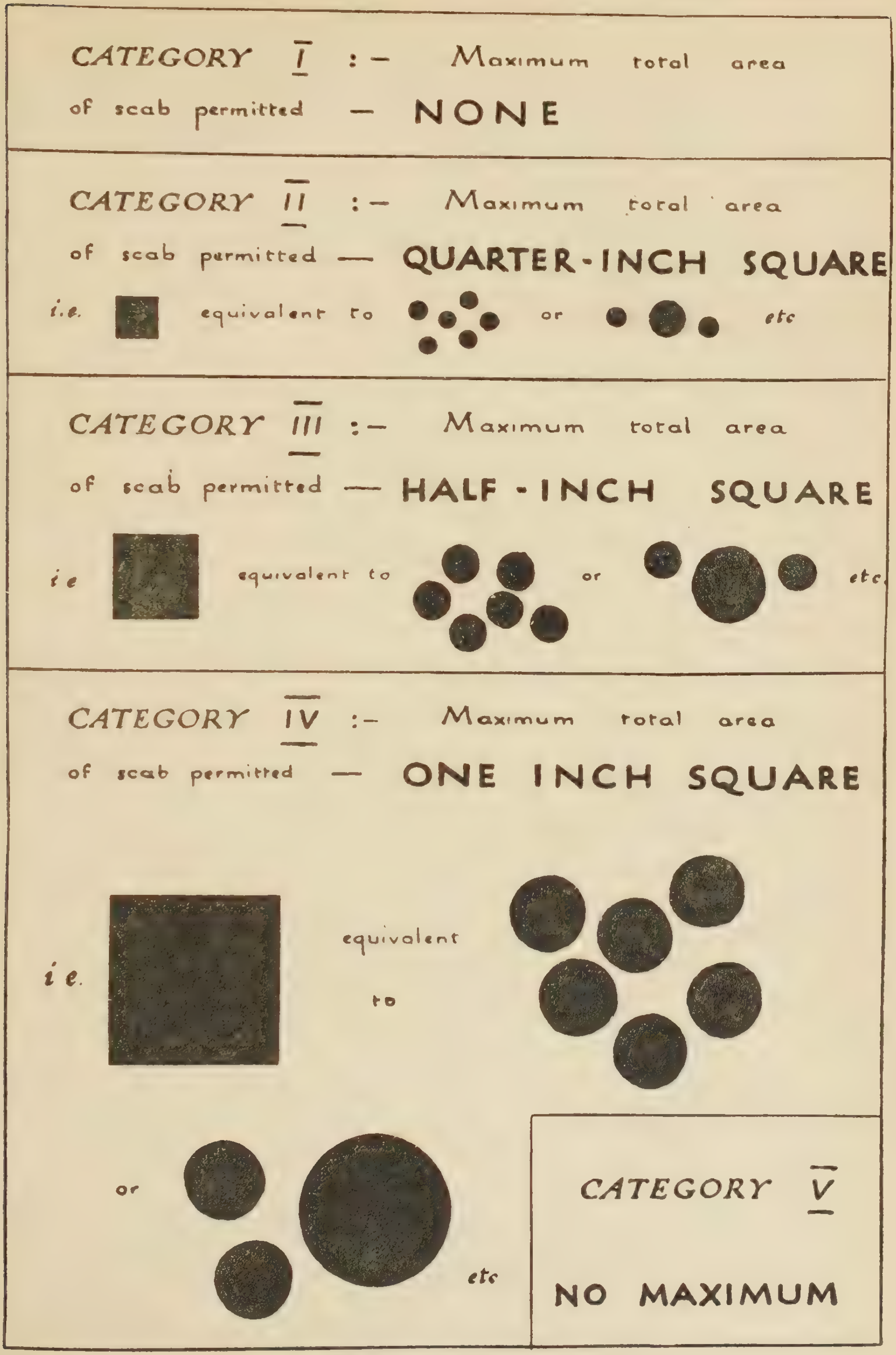
* In *Annals of Applied Biology*, XVII, pp. 419-424. 1930.

SCALE EMPLOYED IN RECORDING DEGREE OF SCAB INFECTION ON FRUIT.



CATEGORY I :— Maximum total area of scab permitted — NONE

CATEGORY II :— Maximum total area of scab permitted — QUARTER-INCH SQUARE

i.e. equivalent to or etc

CATEGORY III :— Maximum total area of scab permitted — HALF-INCH SQUARE

i.e. equivalent to or etc.

CATEGORY IV :— Maximum total area of scab permitted — ONE INCH SQUARE

i.e. equivalent to

or etc

CATEGORY V

NO MAXIMUM

Figure 2

Data on the costs of spraying are given in an appendix.

In many instances, the records are incomplete, due principally to the distance of the centres from the Research Station and the consequent difficulty of making frequent visits.

A summary of the spray trials on the trees of Worcester Pearmain at Bridgwater, Yeovil and Hereford is presented graphically in Fig. 3. The results of the final counts are illustrated in Fig. 4.

Centre 1.—*Bridgwater.*

Spraying was carried out at this centre both in an eight-year-old cultivated plantation and in a thirteen-year-old grass orchard. The orchard comprised approximately 3 acres of Worcester, Cox, Derby and other varieties, while the plantation covered about 15 acres in which Worcester, Cox, Newton, Lane and Bramley were interplanted. The trees stood at 15 feet on the square and averaged 9 feet in height and 8 feet spread. The total number of Worcester trees was 250 in the plantation and 120 in the orchard ; of Cox, 400 in the plantation and 200 in the orchard.

No control measures against scab were carried out in 1929 and the crop of Cox and Worcester in that year was heavily attacked, while the Lanes and Newtons were slightly scabbed. Examination in early April, 1930, showed that scabbed wood was plentiful on Cox and Worcester.

The spraying equipment available was a 3½ h.p. power sprayer and four lances ; the water supply was adjacent to the orchard but about half a mile from the plantation. In drawing up a spraying programme, three applications were recommended for Cox and Worcester while a single application was suggested for Lane and Newton ; the last-named was to be made before blossoming, to avoid spray damage. Bordeaux mixture could not be used for the trees of Cox, so lime-sulphur was employed throughout, to simplify the programme. The complete schedule of applications was, therefore, as follows :—

At green flower stage ..	1 in 30 lime-sulphur (on Cox and Worcester).
At pink stage	1 in 30 lime-sulphur (on Cox, Worcester, Lane and Newton).
At petal fall	1 in 60 lime-sulphur (on Worcester). 1 in 100 lime-sulphur (on Cox).

A block of four control trees was selected in the eight-year-old Cox's.

SUMMARY OF SPRAYING TRIALS ON VARIETY WORCESTER PEARMAIN
AT BRIDGWATER, YEOVIL AND HEREFORD.

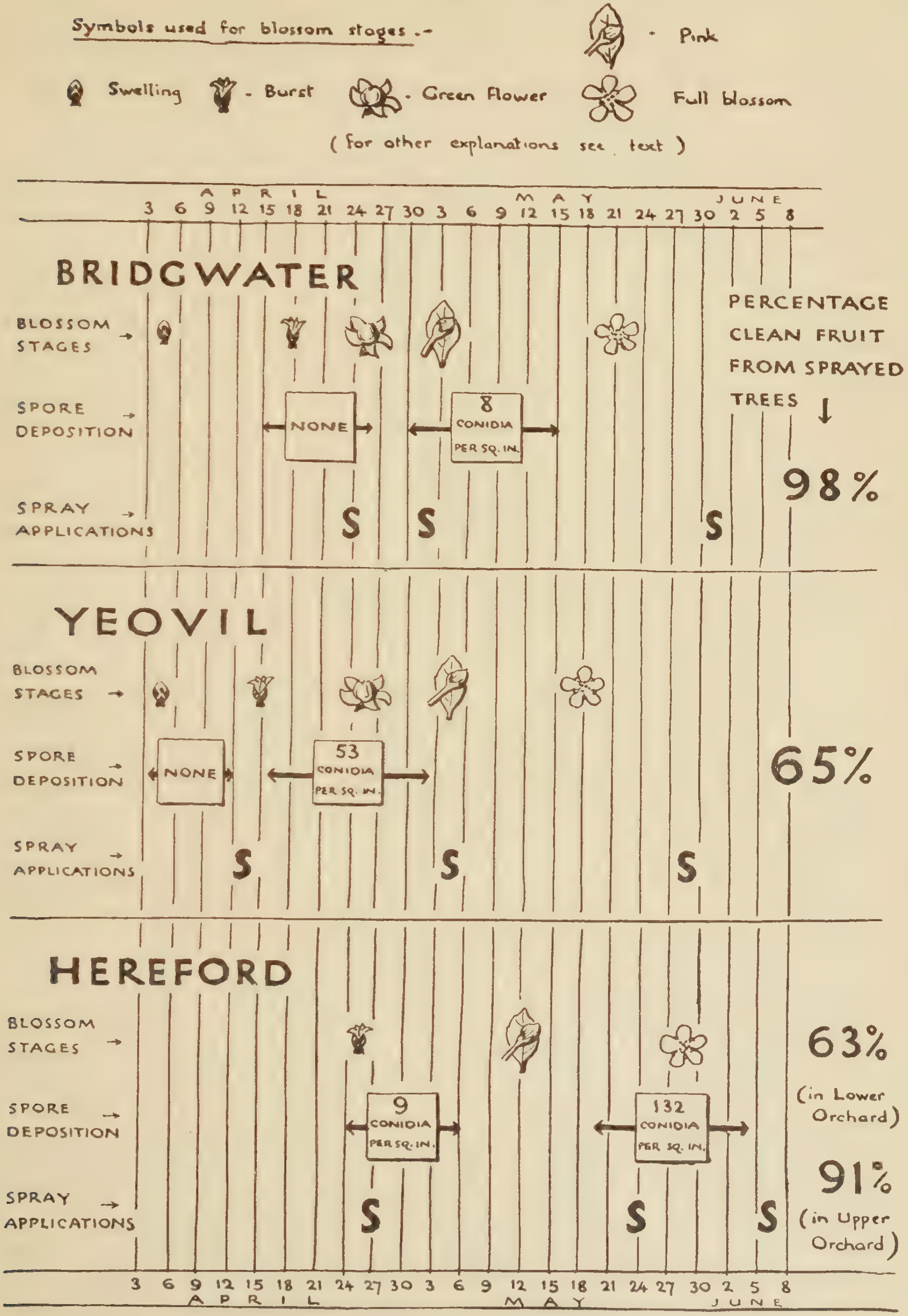
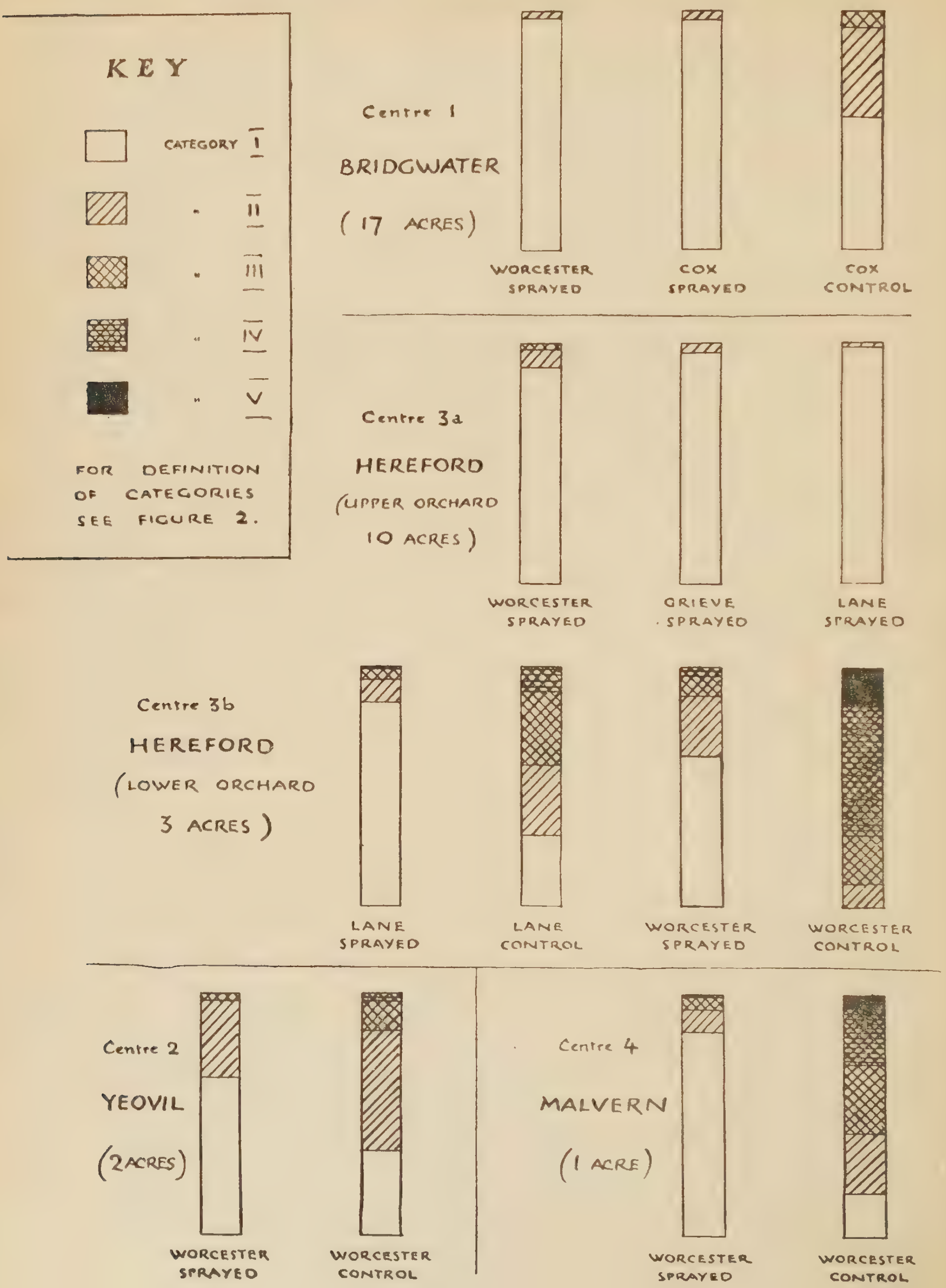


Figure 3

RESULTS OF SPRAYING TRIALS.

DIAGRAMS REPRESENTING AMOUNT OF SCAB INFECTION AT PICKING TIME



Bud development in the spring of 1930 was two to three weeks later than usual. The records were :—

		Worcester.	*Cox.*
April 4	..	Swelling.	Bursting.
,, 18	..	Burst.	Green flower.
,, 25	..	Green flower.	
,, 28	..		Pink.
May 3	..	Pink.	
,, 22	..	Full blossom.	Full blossom.

In the attempt to determine spore deposition, the first series of slides was exposed on the control trees from April 15th to April 26th, but these on examination showed no spores. The next series remained on the trees from April 30th to May 15th. Counts on these slides gave the following results :—

1½ sq. in. of Slide 1	..	9 conidia	..	0 ascospores.
1½ sq. in. of Slide 2	..	15 conidia	..	0 ascospores.

Average concentration after 14 days' exposure—8 conidia per sq. in.

The first spraying, using 1 in 30 lime-sulphur, was started on April 15th on the eight-year-old trees of Cox. The thirteen-year-old Cox's were sprayed on the following day and the Worcesters a week later. The first two days of spraying were gusty but dry.

Lime-sulphur at 1 in 40 was used for the " pink " stage spray, carried out on April 29th to 30th on Cox, Lane and Newton, and May 2nd on Worcester. Slight leaf-tip scorching on Cox was noted following this application.

The post blossom spray was applied on June 2nd, 3rd and 4th. Lime-sulphur at 1 in 66 was used on Worcester and at 1 in 100 on Cox. Very slight leaf-tip scorching was the only damage noted as a result of this spraying.

Scab was first recorded on May 22nd, at which date it could easily be found on the unsprayed trees, an occasional blossom truss being heavily attacked. On the sprayed trees no scab was found at this time. By June 6th, the control trees each showed twenty to thirty scabbed leaves, while of forty sprayed trees (Cox and Worcester) examined at random, thirty-seven were perfectly clean, two showed one scabbed leaf and the other showed three scabbed leaves. At this date the crop on the control trees was much lighter than the average on the corresponding sprayed Cox's.

Counts were made on August 27th on Cox and Worcester only, the Lanes having failed to crop. The yield from the four control trees of Cox was so small that the total number of fruits did not reach 100. For this reason the " percentages " below relating to the control trees are given in brackets. The sprayed Cox's carried a fair crop, amounting to about 12 tons in all ; the Worcesters carried a good crop totalling approximately 14 tons. Samples were taken from the plantation only, but a rough count showed that the results obtained in the orchard were of the same order.

TABLE I.

Results at Bridgwater.

Scab Category.	Variety : Cox.			
	Sprayed.		Unsprayed.	
	No.	Percentage.	No.	Percentage.
I ..	150	97%	36	55%
II ..	4	2%	23	35%
III ..	1	1%	7	10%
IV ..	0	—	0	—
V ..	0	—	0	—
	Variety : WORCESTER.			
I ..	134	98%		
II ..	3	2%		
III ..	0	—		
IV ..	0	—		
V ..	0	—		

Centre 2.—Yeovil.

An area of approximately 1 acre was included in the spraying trial, comprising fourteen rows of trees with thirty-eight trees per row, planted on the square at 10 feet in 1915. From west to east the first four rows were Worcesters, the next five were Cox, and the remaining five were King of the Pippins. These trees had been grassed down and neglected for many years so that they were very stunted, the average height being 8 feet and the spread 6 feet. Following an application of nitrate of soda, the trees in 1929 bore a heavy crop which was completely ruined by scab. In March, 1930, the wood on all three varieties was scabbed very severely.

The spraying outfit available was a barrow pump and two lances ; water had to be hauled about 300 yards.

The spraying programme was drawn up on the same lines as that for the Bridgwater centre, i.e. :

At green flower stage ..	1 in 30 lime-sulphur (on Cox, Worcester and King).
At pink stage	1 in 30 lime-sulphur (on Cox, Worcester and King).
At petal fall	1 in 60 lime-sulphur (on Worcester and King). 1 in 80 lime-sulphur (on Cox).

A block of twenty-eight trees was left as control—eight trees of Worcester and ten each of Cox and King.

The following records of flower-bud development were made :—

		Worcester.	*Cox.*	*King.*
April	4	Swelling.	Bursting.	Bursting.
,,	15 (approx.)	Burst.	Green flower.	Green flower.
,,	25 (approx.)	Green flower.	Pink.	Pink.
May	5	Pink.		
,,	17	Full blossom.	Full blossom.	Full blossom.

(The Cox's and Kings showed considerable variations between one tree and another, especially in the early stages of bud movement.)

The first series of slides was exposed on the control trees from April 4th to 12th. No scab spores were found on these when examined. The next series was exposed April 16th to May 5th : the results of examination of this series were as follows :—

1½ sq. in. of Slide 1	..	33 conidia	..	0 ascospores.
1½ sq. in. of Slide 2	..	148 conidia	..	0 ascospores.
1½ sq. in. of Slide 3	..	97 conidia	..	0 ascospores.
1½ sq. in. of Slide 4	..	43 conidia	..	0 ascospores.

Average concentration after 19 days' exposure—53 conidia per sq. in.

The first spraying, using lime-sulphur at 1 in 30, was started on April 4th when 135 trees of Cox and sixty of King of the Pippins were sprayed. The rest of the trees of these varieties were sprayed on the 10th ; all the Worcesters were sprayed on the 14th. Generally speaking, the buds were at the " burst " stage at the time of spraying—a considerably earlier stage than that suggested in the spray programme for this centre. Except for a light shower on the 4th, the weather conditions were favourable for spraying.

The second spraying of the Cox's and Kings was carried out on May 2nd and of the Worcesters on the 5th. The final spraying took place on May 29th. These sprays were carried out at the stages suggested in the programme (see below). No spray damage resulted from either application.

On May 22nd the control trees were found to have leaves of occasional blossom trusses badly scabbed. No scab was found on the sprayed trees on this date, but by June 12th it was appearing on the leaves of the sprayed Worcesters and Cox's. It was noted that the grass in the orchard was not cut until June 5th, by which time it was covering the lower branches of many of the trees. On July 7th, the leaves of the control trees were literally black with scab while the crop was small and about 30 per cent. marked with scab. The sprayed trees at this date showed approximately 10 per cent. of the fruit scabbed.

Counts were made on August 26th on the Worcesters only, there being practically no crop on the Cox's and Kings. The total yield of Worcesters was approximately 2 tons.

TABLE II.

Results at Yeovil.

Scab category.	Variety: WORCESTER.			
	Sprayed.		Unsprayed.	
	No.	Percentage.	No.	Percentage.
I	273	65%	57	37%
II	137	32%	74	48%
III	13	3%	19	12%
IV	0	—	2	2%
V	0	—	1	1%

Centre 3.—*Hereford.*

This orchard, grassed down in 1929, covered approximately 12 acres, sloping fairly steeply to the south. The trees were planted on the square at 12 feet intervals and were from 8 to 12 feet high and 6 to 15 feet spread. The lower part of the orchard consisted of 3 acres (900 trees) of Worcesters and Lanes alternating ; the Lanes in particular had made vigorous growth and the branches of adjacent trees were commonly interlaced. This block will be referred to below as the " Lower Orchard." The remainder (" Upper Orchard ") consisted first of 200 Newtons, followed by 6 acres (1,800 trees) of Worcesters and Grieves alternating. The Grieves had had much wood cut out following canker attack, so that this part of the orchard was much less dense than the Lower Orchard. The top of the slope carried another 3 acres of Worcesters and Lanes alternating, this block being again relatively open.

Two lime-sulphur sprayings were carried out in 1929, one at the pink blossom stage and one at petal fall, but in spite of this the Grieves, Worcesters and Lanes were all very heavily attacked. In March, 1930, abundant scabbed wood was present on these varieties.

The spraying equipment available was a headland sprayer carrying two guns, and also two knapsack sprayers. Water was laid on to the plantation but at low pressure.

There being no trees of Cox to be sprayed, it was possible to use Bordeaux mixture for all the pre-blossom spraying. Bordeaux could not be used in the post-blossom stage, because of its russeting effect on Grieves, and the Lanes and Newtons were left entirely unsprayed after blossoming because of their susceptibility to damage.

The spraying programme was therefore drawn up as follows :—

Green flower stage ..	Bordeaux 4-10-50 (on Worcester, Lane and Grieve).
Pink stage	Bordeaux 4-10-50 (on Worcester, Lane, Grieve and Newton).
Petal fall stage ..	Lime-sulphur 1-60 (on Worcester and Grieve).

A small block of six controls (three Worcesters and three Lanes) was left in the Lower Orchard.

The records of bud development taken were as follows :—

	Worcester.	*Lane.*	*Grieve.*	*Newton.*
April 25	Burst.	Green flower.	Green flower.	Burst.
May 12		Pink.	Pink.	
June 4	Petal fall.	Petal fall.	Petal fall.	Petal fall.

Slides were placed in the control trees on April 25th and collected about May 8th. Examination showed the following spore deposition :—

$1\frac{1}{2}$ sq. in. of Slide 1 .. 15 conidia .. 0 ascospores.
$1\frac{1}{2}$ sq. in. of Slide 2 .. 12 conidia .. 0 ascospores.
Average concentration after 14 days' exposure—9 conidia per sq. in.

A second series of slides was placed in position on May 19th, and not collected until June 3rd, by which time scab on the newly-formed leaves was plentiful. The consequence was, as might be expected, that the concentration of spores on the slides was very high.

$1\frac{1}{2}$ sq. in. showed 198 conidia .. 0 ascospores.
Concentration after 14 days' exposure—132 conidia per sq. in.

The first spray (Bordeaux 4-10-50) was applied to Worcesters, Grieves and Lanes during the period April 22nd to 26th and 28th. The Worcester buds were approximately at the "burst" stage; those of Grieve and Lane had nearly reached the "green flower" stage. Spraying was interrupted by rain on the 23rd and 25th, but there were no strong winds.

The second spray at the same strength was carried out on Worcester, Grieve, Lane and Newton. This spraying continued from May 16th to 24th and was only just completed before the blossoming stage.

The third spraying, using lime-sulphur at 1 in 60 strength was made on Worcesters and Grieves only on June 4th, 5th and 6th. An examination for spray damage on July 3rd showed that a few of the spur leaves of Worcester had fallen, and that a faint diffuse russetting was common on the fruits of Worcester and Grieve. It is possible that this russetting may not have been the effect of the lime-sulphur but of the very late second pre-blossom spray with Bordeaux.

The first examination for scab was made on June 3rd, on which date the unsprayed Worcesters showed about twelve badly-scabbed leaves per tree. A month later, the sprayed trees were still completely free from scab, but on the unsprayed Worcesters, of 100 fruits counted, fifty-two were seriously scabbed. At this date (July 3rd) the unsprayed Lanes showed abundant scabbed foliage but no scabbed fruit. The further development of a heavy scab attack on the crop of these trees is shown by the counts below.

Final records were taken on August 29th and, owing to the marked differences observed between the results in the Upper and the Lower Orchards, these are shown separately.

TABLE IIIa.

Results at Hereford (Upper Orchard).

Variety :	WORCESTER.		GRIEVE.		LANE.	
Scab Category	Sprayed.		Sprayed.		Sprayed.	
	No.	Percentage.	No.	Percentage.	No.	Percentage.
I	137	91%	303	96%	300	98%
II	11	7%	12	4%	4	1%
III	2	1%	0	—	4	1%
IV	1	1%	0	—	0	—
V	0	—	0	—	0	—

TABLE IIIb.

Results at Hereford (Lower Orchard).

Scab Category.	Variety : WORCESTER.			
	Sprayed.		Unsprayed.	
	No.	Percentage.	No.	Percentage.
I ..	82	63%	0	0%
II ..	32	25%	8	8%
III ..	13	9%	22	21%
IV ..	3	3%	55	54%
V	0	—	17	17%
	Variety : LANE.			
I ..	122	86%	41	29%
II ..	13	9%	43	30%
III ..	5	4%	45	31%
IV ..	1	1%	12	9%
V ..	0	—	1	1%

Centre 4.—*Malvern.*

The spraying at this centre was carried out on approximately 1 acre of a twenty-two-year-old plantation of Worcesters, growing under conditions of clean cultivation. The trees had been planted on the equilateral triangle system at 18 feet intervals and measured about 12 feet high by 15 feet spread. The block included in the spraying trial comprised 139 trees, of which six were left as control. This trial differed from all the others in that the trees employed had been given adequate spraying against scab in the previous season. The crop in that year was very light but 95 per cent. scab-free. Extensive pruning was carried out in the winter of 1929-30 and at the end of March little scabbed wood could be found. No attempts to measure spore deposition were made at this centre.

The equipment available was a power sprayer (4 h.p.) and four lances. Water had to be hauled about 300 yards to the plantation.

With the object of repeating the 1929 trial, the spraying programme recommended was :—

At green flower stage	..	Bordeaux 4-8-50.
At petal-fall stage	..	Bordeaux 2-4-50.

The only records of bud development taken were as follows :—

			(Variety—*Worcester*.)
April	9	..	Swelling.
,,	24	..	Burst.
June	3	..	Petal-fall.

The first spraying was carried out on April 24th, in calm, cloudy weather. A light rain fell about four hours after spraying. The date of the post-blossom application was June 9th : on this day the weather was sunny with a wind of 10 to 15 m.p.h. A month later, the sprayed fruits showed occasional russetting at the stalk end but no cracking resulted.

An inspection on June 3rd revealed no scab either on the controls or on the sprayed block. By July 2nd, however, scab was easily noticeable on the unsprayed Worcesters, while the sprayed block showed only one or two scabbed fruits per tree. A count made on August 2nd showed that the fruits on the control trees were 39 per cent. scabbed while those on the sprayed trees were only 5 per cent. scabbed. The final counts, taken on a good crop on August 28th, showed the following results :—

TABLE IV.

Results at Malvern.

Scab Category	Variety : WORCESTER.			
	Sprayed.		Unsprayed.	
	No.	Percentage.	No.	Percentage.
I ..	273	84%	40	19%
II ..	30	9%	51	24%
III ..	21	6%	58	28%
IV ..	2	1%	51	24%
V ..	0	—	10	5%

DISCUSSION.

The spraying programmes recommended proved quite practicable on a commercial scale and were carried out without serious difficulty although the spring was unusually showery. The results obtained were highly satisfactory at Bridgwater and in the Hereford Upper Orchard, i.e. on a total of approximately 25 acres of Worcester, Cox, Grieve, Lane and Newton. From this acreage, in spite of a wet summer, the crop obtained was from 91 to 98 per cent. entirely free from scab. In the remaining portions of the trials—3 acres of Worcesters and Lane in the Hereford Lower Orchard and ⅓ acre of Worcesters at Yeovil—the results did not attain the same standard, but considering the intensity of the scab attack in these areas the effectiveness of the spray treatment was striking. Thus, at Yeovil, the unsprayed Worcesters were 37 per cent. scab-free, the sprayed 65 per cent. In the Hereford Lower Orchard the unsprayed Worcesters had no scab-free fruits ; the sprayed had 63 per cent. The reason for the heavy scab attack at Hereford lay undoubtedly in the fact that the dense interlacing trees in the Lower Orchard provided conditions highly favourable to the fungus ; at Yeovil, the long grass may have acted in a similar way. The possible relation of the time of spraying to the progress of infection at these centres is dealt with below.

The results of the experiments on spore deposition were rather surprising. It was expected that both over-wintered leaves and scabbed twigs would be found acting as sources of infection, but, during April and May, the only source of scab infection demonstrated was the pustules on the wood. A total of over 500 conidia was counted at different times on portions of the various slides exposed, but not a single ascospore was recorded on any slide. In considering this result it must be borne in mind that the technique employed may have favoured the retention and the visibility of the conidia more than the ascospores and, again, that the winter of 1929-30 was extremely favourable to the complete destruction of fallen leaves. Nevertheless, it appears that the hypotheses, based on the work of Keitt* and others in the U.S.A., concerning the importance of infection from the over-wintered leaves, ought not to be accepted for this country without further examination. Additional evidence pointing to the importance of scabbed wood as a source of primary infection in the spring has frequently been obtained during the 1930 season by noting the proximity of the first-scabbed leaves to active scab pustules on the twigs.

The time at which conidia was first caught on the slides varied in the different plantations. At Bridgwater none was caught until the fortnight April 30th to May 15th. At this centre the buds of Worcester were at the pink stage on

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* G. W. Keitt and L. K. Jones. Studies of the Epidemology and Control of Apple Scab. *Wisconsin Agric. Exper. Stat. Research. Bull.* 73. 1926.

May 3rd. At Yeovil, where the dates for the different stages of bud development were approximately the same as at Bridgwater, no conidia were caught before April 12th, but fifty-three conidia per square inch were recorded in the period April 16th to May 5th. At Hereford, where the trees were about ten days later, nine conidia per square inch were caught between April 25th and May 8th. Thus, at Yeovil and Hereford, the deposition of conidia was well marked during the fortnight *before* the buds reached the pink stage, but in none of the centres was there any record of deposition of conidia until after the " burst " stage. The recommendation to give a first spraying at or about the green flower stage thus appears to be justified. If spraying is done before this stage, very few or no conidia are falling on to the young leaves, and the amount of the leaf surface exposed to the spray is very small. As the leaves expand still further, the tips only remain protected by the spray and the rest of the leaf is left vulnerable to infection until the time that the second spray is applied.

Turning to the results of the 1930 trials (and see Fig. 3) it will be noted that at the Yeovil and Hereford centres the first sprayings were applied at approximately the " burst " stage on Worcester (although at the correct stage for the other varieties). In the Upper Orchard at Hereford, where the trees were not crowded and the conditions for scab development therefore not so favourable, this too-early spraying was not markedly prejudicial to scab control. But where conditions for scab infection were more favourable, as in the Hereford Lower Orchard and at Yeovil, the long gap between the first and second spraying of the Worcesters gave the opportunity for early leaf infections to occur. In the wet period from mid-July onwards infections from the leaf pustules thus initiated had an excellent opportunity for passing on to the fruit.

The trial at Bridgwater was not strictly comparable with those at Yeovil and in the Hereford Lower Orchard, since the records of spore deposition and of the infection of the control trees show that the intensity of scab attack was here much lower. But it is probably not solely due to this fact that, at this centre, where the times of application were strictly at the green flower, pink and petal-fall stages, the results obtained were uniformly excellent.

The Malvern trial has not hitherto been considered and stands in a class by itself, since this was the one centre at which scab had been successfully controlled in the previous year. In the writer's opinion it was this 1929 spraying, more than the premature first spray of 1930 which, by eliminating scabbed wood, kept the leaves clean up to blossom time. Where trees have been practically freed from scab in one year it is possible to take considerable liberties with the spraying programme in the following year and still obtain satisfactory results.

The costs of spraying will not be materially different, whether the bulk of the spraying is done after blossoming or before, but the question of sparing the labour is frequently more acute after midsummer. The possibility of completing the scab control programme by June is particularly attractive in fruit plantations where the acreage is made up not only of apples, but also of soft fruit. For such plantations, labour for scab spraying is rarely available after the commencement of strawberry picking. But the ability to carry out a scab control programme in the time between the green flower and the petal-fall stages depends, where the apple acreage is large, upon making the fullest use of favourable weather periods for scab spraying. Insufficient spraying equipment, or the necessity for applying an additional insecticidal wash in the spring are the two factors most liable to upset a scab spraying programme. The degree of control of insect pests obtained during the winter is, because of its effect on the time available for spring spraying, a factor of considerable importance in scab control.

SUMMARY.

The possibility of controlling apple scab by two pre-blossom and one post-blossom sprays was examined by means of field trials in Somerset, Worcestershire and Herefordshire.

Excellent control was obtained when sprays were applied at the "green flower," "pink," and "petal-fall" stages. Where the first spray was applied before the "green flower" stage the control was less effective.

It was shown by exposing vaselined slides that conidia from wood pustules were present in the air around trees of Cox and Worcester at the time that the first leaves were unfolding. No ascospores (from over-wintered leaves) were caught on these slides. The relation of the time ot spraying to the presence of infecting spores is considered.

The costs of the spray treatments are given in certain instances and the practical advantages of early spraying are discussed.

Diagrams are given illustrating a method of estimating scab damage and a nomenclature for blossom bud stages.

APPENDIX—SPRAYING COSTS.

Data concerning spraying costs (see opposite page) were obtained for the following spray applications:—

Centre 2.	Yeovil ..	Both pre-blossom applications.
Centre 3.	Hereford ..	First pre-blossom application.
Centre 4.	Malvern ..	One pre-blossom and one post-blossom application.

COSTS FROM 1930 FIELD TRIALS.

Date.	Centre.	Machine.	Nozzles.	Spray Fluid.	Gallons.	Cost of Materials.	Spraying Man-hours.	Attendant Man-hours.	Layout Man-hours.	Labour Cost (at 10d. hour).	Total Nett Cost.	Water-carting Cost.	Waste Time Cost.	Gross Cost.	Nett per tree.	Nett per Acre.	Remarks.
						£ s. d.				£ s. d.	£ s. d.	s. d.	s. d.	£ s. d.	d.	£ s. d.	
April 4, 10, 15	Yeovil	Barrow pump	2 lances	L.-S. 1-30	255	12 9	19	9½	2½	1 5 10	1 18 7	6 5	2 1	2 7 1	0.9	1 11 0	
May 4	ditto	ditto	ditto	ditto	270	13 6	16	8	1½	1 1 3	1 14 9	4 7	1 3	2 0 7	0.8	1 7 8	
April 22-26 & 28	Hereford	Head-land & 2 knap-sacks	2 guns 2 lances	Bdx. 4–10–50	4,000	5 16 0	160	120	20	12 10 0	18 6 0	—	16 8	19 2 8	1.2	1 9 10	Interruptions by rain.
April 24	Malvern	4 h.p.	4 lances	Bdx. 4–10–50	420	14 2	16	5	2	19 2	1 13 4	11 4	1 8	2 6 4	3	1 13 4	Application very thorough.
June 9	ditto	ditto	ditto	Bdx. 2–5–50	360	6 0	14	4	1	15 10	1 1 10	11 4	10	1 14 0	2	1 1 10	

The data received refer to the following blocks of trees :—

YEOVIL ..	522 bush apples in grass, average 8 feet height, 6 feet spread, planted at 10 by 10 feet	Area 1¼ acres.
HEREFORD ..	3,600 bush apples in grass, 8 to 12 feet height, 6 to 15 feet spread, planted 12 by 12 feet	Area 12 acres.
MALVERN ..	133 half-standard apples cultivated, 12 feet height, 15 feet spread, planted on triangular system at 18 feet intervals	Area approx. 1 acre.

For ease of comparison, the figures are presented in the same form as that given by Maynard in Long Ashton Annual Report, 1929, p. 175.

(*Received January*, 1931.)

RASPBERRY CANE SPOT: ITS DIAGNOSIS AND CONTROL.

By R. V. HARRIS,
(*East Malling Research Station*)

I. GENERAL INTRODUCTION.

In June of 1924 the writer, while examining the large collection of raspberry varieties at the Research Station, noticed that the stems of the young canes of certain varieties were heavily infected with minute spots, which later in the season were observed to develop into small pits. Towards the close of the growing season the growth of cane of the varieties most heavily infected with the spotting had been seriously impeded and the upper portion of many canes was dead. In 1925 the contrast between the vigour of growth of the young cane and that of the fruiting cane was very marked, particularly in the case of the most severely spotted variety—*Baumforth Seedling B,* the fruiting cane of which was early overtopped by the young cane, and the fruit crop in 1925 markedly reduced.

That this reduction of the vigour of the cane and of the crop was related to the spotting was very evident, and it was decided to investigate the disease further with a view to applying control measures. In May, 1925, a very slowly-growing fungus was isolated from lesions on a fruiting cane and was found to correspond closely with descriptions of the fungus *Plectodiscella veneta* (Speg.) Burk. by Burkholder, who showed also by inoculation that it was the cause of the Anthracnose disease of species of Rubus in America (4). In 1925 the conidial or *Gloeosporium* stage was observed on cane lesions and on pure cultures of the fungus isolated from the canes. In the spring of 1926 the ascigerous or *Plectodiscella* stage of the fungus associated with the lesions, was discovered on canes at East Malling, and its connection with the conidial or *Gloeosporium* stage of the fungus was determined by cultural methods. Later in that season inoculation experiments were carried out with pure cultures of the fungus obtained from a single ascospore, and in one experiment the leaf-spot stage of the disease was reproduced. During this season also, preliminary experiments in the control of the disease (1) by a modification of the customary cultural practices and (2) by spraying, were carried out. The latter experiment was based on the American work on Anthracnose control on the Black Raspberry (4,21 etc.), and on field observations at East Malling. The degree of control obtained in both these experiments and by both the fungicides used was very satisfactory, and as a result a preliminary report on the control of the disease was published in 1928 setting out the results of the 1926 experiments and suggesting tentatively a spraying schedule for the use of growers (17).

The results of further spraying experiments in 1928 and 1929 indicated, however, that this schedule must be somewhat modified.

In America the disease has been recorded on the two American species of Red Raspberry (*Rubus idæus* L. and *R. idæus* var *aculeatissimus* (Mey.) Regel and Tiling), the Black Raspberry (*R. occidentalis* L.),—the Purple-cane Raspberry, the Loganberry,—the Blackberry and the Dewberry (4). The disease is most serious on the Black Raspberry and the greater part of the American work deals with the control of Anthracnose on this host. Serious attacks have been reported on the blackberry by Rees (28) and by Dodge and Wilcox (12), who report considerable damage to dewberries in North Carolina.

In Great Britain the present author has identified the disease and isolated the causal fungus from Blackberry and Loganberry in addition to the Red Raspberry. Whereas serious attacks have not been observed on the blackberry, considerable damage to the bines is evident in numerous Loganberry plantations on the Weald of Kent.* This paper is, however, limited to a description and discussion of the disease as it affects the European Red Raspberry. Its attacks on certain varieties of this raspberry are so constant and serious that if the growing of such varieties is to be profitable, the application of routine measures to control this disease is a matter of necessity.

In America the disease is known as *Anthracnose*. The present writer, in suggesting the name " *Cane-spot* " for the disease as it occurs in Great Britain, does so simply with the desire to fix a name which will suggest the salient symptoms of the disease to the practical grower.

II. SYMPTOMS OF THE DISEASE.

A. On the New or First Year Canes.

(1) *The Stem.*

Minute circular purple spots appear on the surface of the canes, usually at first near the base, from the latter part of May onwards, and in some seasons not till the middle of June. These spots measure from 0.5 to 1.0 mm. in diameter and as the cane grows, increase in size and become elliptical in outline, the major axis being parallel to the main axis of the cane. The centre of the spot also loses its purple colour, and becomes silvery white. Thus canes examined in the middle of June, 1929, showed incipient circular spots and also a number of elliptical spots (major axis not exceeding 2 mm. in length) with minute whitish centres (Plate I., Fig. 1). Later the whitish centres encroach

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* The attack of the disease on loganberries in 1930 (affecting the crop of 1931), was of an extremely widespread and serious nature. Experiments in control by spraying are in progress at East Malling.

PLATE I.

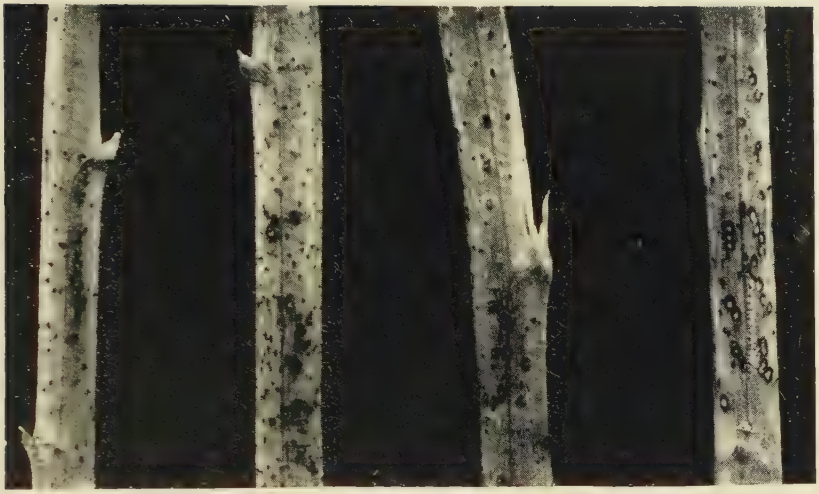

FIG I.
Young canes showing incipient spots. (June, 1926.) Natural size.

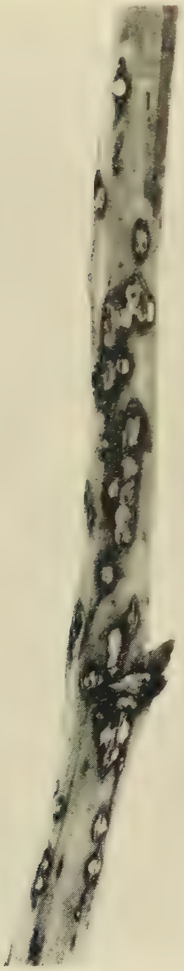

FIG. 2.
A New Cane (var. *Readers Perfection*) showing pit
(September, 1926.) Natural size.

FIG. 3.
A badly cankered fruiting cane (var. *Semper
Fidelis*). Natural size.

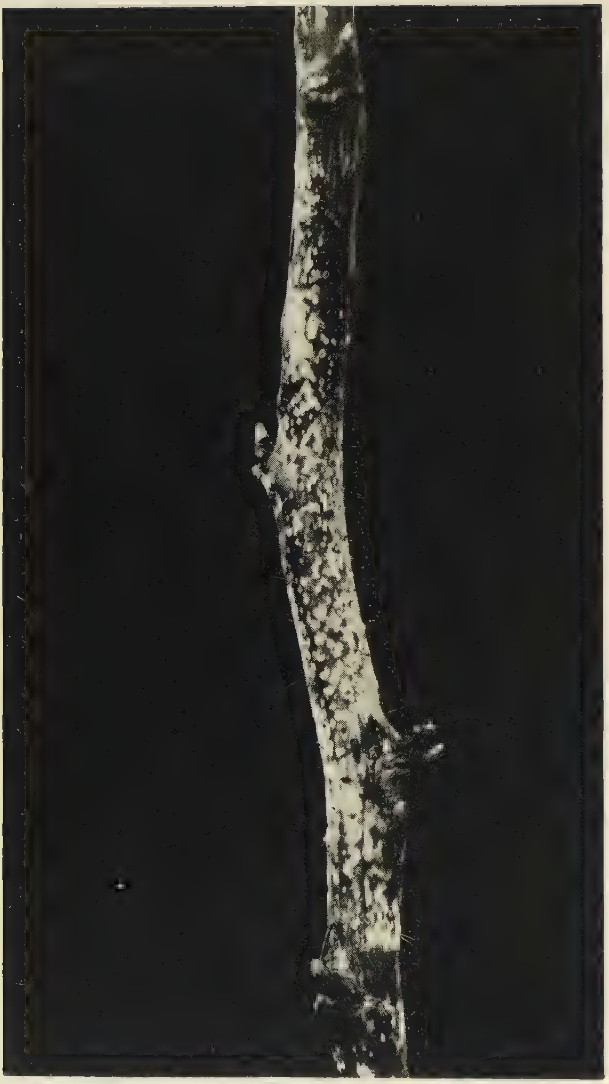

FIG. 1.

A new cane (var. *Semper Fidelis*), showing "superficial" spots. (November, 1925.) Natural size.

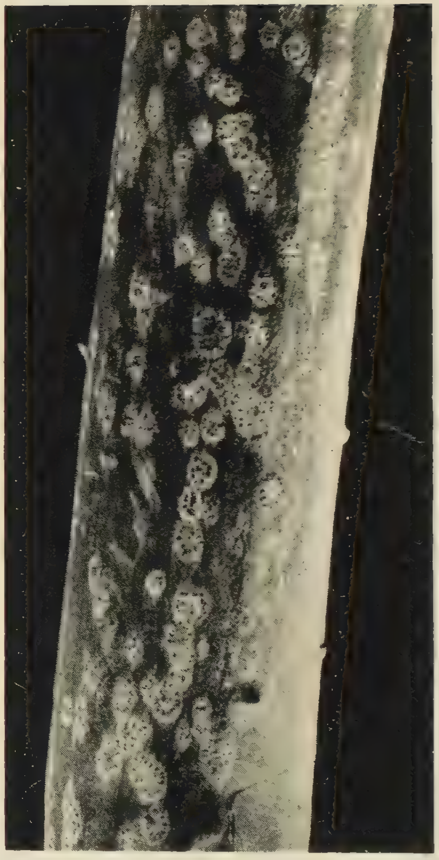

FIG. 2.

A fruiting cane (var. *Readers Perfection*) showing fungal fructifications on "superficial" spots. (April, 1928.) x 4.

till only a narrow purple margin is left. Spots on canes examined on July 1st, 1925, measured up to 3 mm. in length, and the white centre, which at this stage was very slightly raised above the surface of the cane, was found on microscopical examination to be of the nature of a stroma, consisting of the host tissues permeated by fungal mycelium.

After this stage the whitish centres slowly become sunken below the surface of the surrounding cane until they assume the form of elliptical pits with very narrow purple margins. Pit lesions on a cane of *Semper Fidelis* examined in September, 1926, measured on an average 6 by 2 mm. The actual depression or pit measured 4 mm. by 1.5 mm. (Plate I, Fig. 2). The distribution of these spots over the cane is very rarely uniform ; they generally appear in groups. Where the infection is very concentrated the spots coalesce as they develop and cankers with irregular outlines are formed. Such is the development of the typical spot resulting from the earliest infection of the cane.

Infections of the canes, however, take place at all times during the season from the middle of May till the end of October. As an infection during the growing season results in the killing of a minute area of the cambium, the degree of depth of the resulting pit lesion depends on the time at which infection takes place. Thus early infections produce deep well defined pits and later infections progressively shallower pits.

During the seven seasons (1924-30) in which the disease has been under observation a considerable infection of the canes of susceptible varieties has taken place late in the season, i.e., from the latter part of August onwards. The result of these infections has been the appearance, late in the season, of large numbers of small circular purple spots not larger than 1 mm. in diameter. These spots, unlike the earlier infections, do not grow further in size or become sunken, but as the canes in ripening change from green to brown, they become greyish-white in colour, and from early October onwards, are seen to be covered with minute black dots, just visible to the naked eye and easily seen with a lens (Plate II.). These dots are *acervuli** or incipient fructifications of the causal fungus, on which summer spores or conidia are produced, and from many of them ascospores will be ejected the following spring.

* The following definitions of the purely mycological terms used in this paper are supplied to meet requests from our fruit grower readers.

Mycelium.	The vegetative part of a fungus consisting of fungus threads (*hyphæ*).
Stroma.	A mass consisting of host tissues permeated by fungus *hyphæ*.
Acervulus.	(Latin, a little heap.) A small cushion of fungus tissue which emerges from the host plant and bears spores.
Conidia.	Spores produced directly (i.e., budded off) from the fungus *mycelium* (e.g., from an *acervulus*).
Ascospores.	Fungus spores (produced by a sexual process) in a sac known as an *ascus* (Greek, Ascos, a wine skin) which is contained in a fructification, the *ascocarp*.

These minute, late-appearing, non-sunken spots will be referred to as the " superficial " type of spot, to distinguish them from the better known pit lesion, the result of early infections.

(2) *Leaves and Petioles.*

Any time from the middle of June onwards, spots may be observed on the leaves and petioles. As on the stem the first sign of infection is a minute purple spot which later develops a whitish centre. In the case of leaf infection the spots are very small (not larger than 1 mm. in diameter), and they do not increase in size. They are much smaller than the leaf spots of the similar disease on the loganberry and they tend to aggregate themselves midway between and parallel to the main lateral veins. In cases of serious attack the spots are so confused that it is difficult to distinguish the individual infections (Plate IV, Fig. 2). In cases of serious leaf and petiole infection the laminæ curl downwards and premature defoliation may take place (Plate IV, Fig 3). Such was the case with the variety *Baumforth Seedling B* as a result of the serious attack in 1924.

(3) *The effects of infection on the growth of the new canes.*

It will readily be seen from the above account of the direct symptoms of infection on the new canes, that the chief damage is caused by the early infections (viz., lesions appearing in May and June). The direct damage caused by the " superficial " type of spot would appear to be inconsiderable.

In years of severe early infection the growth of the canes is impeded and premature defoliation of the canes takes place. In 1924 the stunting of the canes and curling of the leaves were noticed as early as June 24th. Where the lesions are very concentrated and coalesce the stem sometimes becomes very twisted and its surface hypertrophied in the neighbourhood of the cankers, although the large knotty growths noticed by Burkholder on the Red Raspberry have not been observed by the author. On September 2nd, 1924, the tips of many of the new canes of *Baumforth B* were found to be dying as a result of severe infection.

B. ON THE FRUITING OR SECOND YEAR CANES.

(1) *Stems.*

In the fruiting year of the canes the cracking of the cankered areas and of the pit lesions increases and the sclerenchyma strands are seen in greater prominence (Plate I, Fig. 3). Owing to the change in colour of the canes from green to brown, the individual pit lesions do not stand out distinctly and the purple margin has disappeared. On the other hand the " superficial " spots

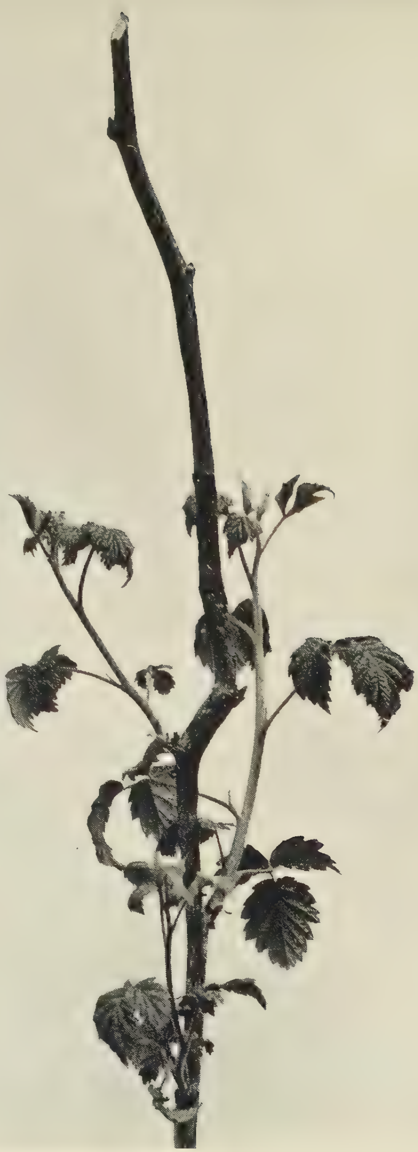

FIG. 1.

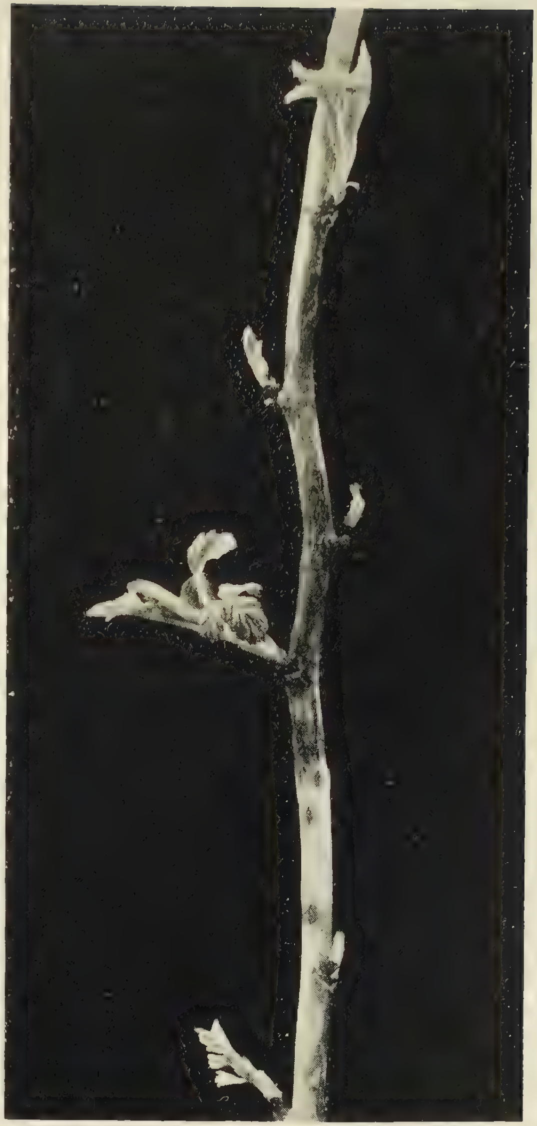

FIG. 2.

PLATE IV.

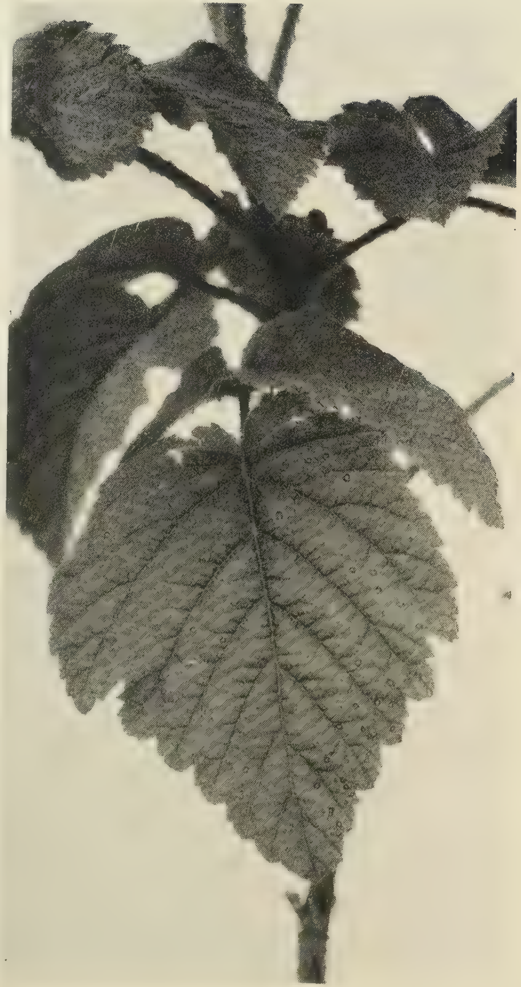

FIG. 1.

A seedling Raspberry plant sprayed with a suspension of conidia of *P. veneta*, photographed four weeks after infection. Note typical spots on the leaf and compare with natural infections shown in Fig. 2. Natural size.

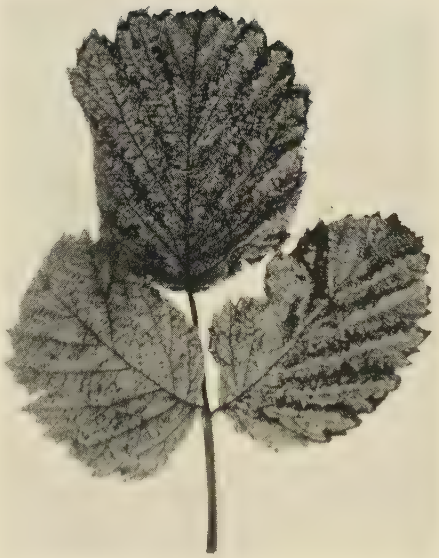

FIG. 2.

A badly infected leaf from a fruiting cane (var. *Baumforth Seedling B*). (August, 1925.) Natural size.

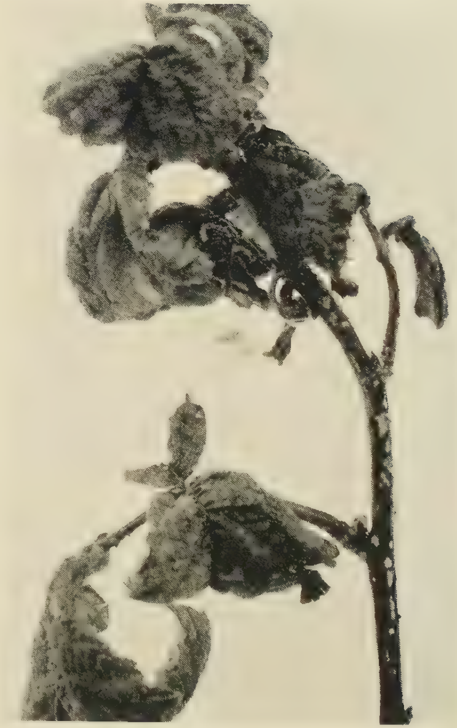

FIG. 3.

The apical portion of a young cane showing a severe infection of the stem, petioles and leaves. Note the downward curling of the latter which immediately precedes defoliation. (August, 1925.) Natural size.

stand out in clear relief and the black dots on the little white discs are more distinct than in the autumn of the first year (Plate II, Fig. 2).

(2) *Leaves and Fruiting laterals.*

The spots on the leaves in size and distribution are similar to those on the leaves of the new cane, but usually appear earlier (i.e., in May). Spots similar in appearance to those on the new canes also appear on the fruiting laterals, but an attack on laterals sufficiently serious in itself to impede their development has not been observed.

(3) *The effect of the disease on the development of the fruiting canes.*

The indirect symptoms of the disease on the fruiting canes are very striking and are visible as soon as the buds begin to grow out.

It is then observed that many of the buds fail to grow out at all or only produce one or two dwarfed leaves ; other laterals grow out but remain dwarfed and do not produce fruit (Plate III, Fig. 2). The apical portion of many of the canes is also seen to be dead (Plate III, Fig. 1).

That these symptoms are a direct result of infection by cane spot will be seen by referring to Table IV. (p. 85), where it will be seen that the reduction of the intensity of the attack of the disease by spraying increases the vigour of growth of the cane and the proportion of buds producing normal fruiting laterals.

III. THE CAUSAL FUNGUS.*

A. ISOLATION AND IDENTIFICATION IN PURE CULTURE.

The isolation of the causal fungus is a matter of considerable difficulty owing to its very slow rate of growth on artificial media. The writer has not succeeded in isolating it directly from pit lesions on fruiting canes, but successful isolations have been made (1) directly from young pit lesions on new canes in July and from young leaf spots, (2) by plating out single acervuli dissected from " superficial " spots on fruiting canes, and (3) by inducing the discharge of ascospores on to culture plates. By all these methods a very slow growing mycelium has been obtained which corresponds to Burkholder's description of *Plectodiscella veneta* in pure culture (4), and the organism is therefore referred to this species. The ascigerous stage of the fungus has not been observed in pure culture. The mycelium, however, which is normally barren may be stimulated to produce conidia of an average size of 6.5 × 3 by transferring it to a very humid atmosphere or vice-versa. These conidia resemble those commonly produced by the fungus on cane lesions, i.e., the summer or *Gloeosporium* spore.

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* A paper dealing in detail with methods for isolating the causal fungus from diseased canes, and its morphology on the canes and in pure culture, is in course of preparation and will serve to supplement the brief account here given.

B. Inoculation Experiments.

Numerous inoculations of raspberry canes from pure cultures of the fungus isolated from lesions on canes have been made, both by the direct transfer of small pieces of mycelium and by spraying the canes with a water suspension of conidia from artificial cultures. In one of these experiments the characteristic leaf-spot symptom of the disease was reproduced (Plate IV, Fig. 1) but in no instance have pit lesions appeared on the stems of inoculated canes. It would appear that the conditions for the production of stem lesions are of a specialized nature. Inoculations with ascospores have not yet been carried out.

C. The Seasonal Cycle of the Fungus on the Canes.

The early stages of the development of the fungus on first year canes have already been described. Summer spores are intermittently abstricted from the stromata and acervuli on lesions, from June till as late as November in this year and in the second or fruiting year they have been found associated with acervuli on " superficial " spots at all times of the season from early February till the cane is dead.

The ascigerous (or ascus-bearing) stage has not yet been observed on first year canes. On fruiting canes it is but rarely that spore-bearing bodies are found on pit lesions (the ascigerous stage has never been found on such lesions). By this time the stromata in these lesions have become shrunken and in many cases have dropped out, leaving simple pits. On the other hand ascocarps develop in large numbers on the minute "superficial" spots (already described) from February onwards. In most seasons it is possible to find mature asci from the end of March onwards. Ascocarps are not easily distinguished from acervuli ; the former, however, are usually larger and more circular in shape than the latter and on crushing them the circular asci are extruded.

IV. THE CONTROL OF THE DISEASE.

A. Resistant Varieties.

During the seasons of 1924 to 1929 a record of the incidence of the disease on the large collection of raspberry varieties at the Research Station was kept and many cases of attack on growers' plantations, in widely separated localities throughout the country, have also been investigated. These records indicate clearly, that varieties differ very markedly in their degree of susceptibility to the disease, and further, that this relative susceptibility remains constant in varying localities and under varying seasonal conditions.

Of the varieties hitherto observed *Baumforth Seedling B* has proved to be outstandingly the most susceptible. At the Research Station the crop of this variety was very seriously impaired by attacks of the disease in the seasons of

1924, 1925, 1926 and 1928 and in the great majority of other serious cases of Cane Spot attack to which the writer's attention has been drawn by advisers and growers, the variety in question has been found to be *Baumforth Seedling B*. It is very evident, as will be seen from the data in the concluding section of this paper, that where this variety is grown commercially the application of direct measures for the control of Cane Spot is a matter of necessity. On no other variety has a greater concentration of spotting been observed but its extreme susceptibility is also probably due in part to the thin whippy nature of its canes. The effect of the pit lesions on the conducting system of canes of this type is greater than the effect of the same number of infections on a variety with stouter canes. The slowly increasing impairment of the size and vigour of the canes by " Mosaic " (to which also this variety is very susceptible) accentuates its susceptibility to Cane Spot. In addition to *Baumforth B*, varieties which are also seriously susceptible, only in a less degree, are, *Semper Fidelis A*, *Readers Perfection*, *Devon*, *Red Antwerp C*, and *Baumforth A*. Attacks of the disease sufficiently serious to justify the application of direct control measures have been observed on all these varieties.

Among varieties upon which cane spotting is only rarely found and which therefore appear to be very resistant to the fungus, *Baths Perfection* and *Pynes Royal* are outstanding examples. In the majority of the other varieties, although canes are commonly infected, the attack has no appreciable effect on their vigour and development. Whether this resistance to the disease will remain constant, or whether, as a result of factors such as the permeation of the variety with " mosaic," it will, in some cases, deteriorate, remains to be seen. At the moment, however, there is a wide range of moderately susceptible and slightly susceptible varieties from which the grower may select and be reasonably confident that the resulting plantation will not suffer seriously from Cane Spot attacks.

B. CULTURAL METHODS.

An experiment was carried out in 1926 to determine the degree of control obtainable (i) by the removal of all the fruiting canes and (ii) by the removal of the most seriously infected fruiting canes, before the new canes appear above ground level in the spring.

A single row, 150 feet in length, of badly infected stools of the variety *Semper Fidelis* was divided by stakes into ten plots, each fifteen feet in length and containing about fifteen stools. Each plot was subjected to one of the following treatments in the early part of March :—

(a) Fruiting canes thinned but the most seriously infected canes *not* cut out.

(b) Fruiting canes thinned and the most seriously infected canes cut out and burnt.

The following are the chief points of interest arising from the results of the single spraying experiment set out in Tables II. to V.

TABLE II.

Summary of records made on June 14th, 1926, showing the effect of 1926 spraying on the incidence of the disease.

Treatment.	No. of stools recorded.	No. of canes recorded.	Percentage of canes in disease categories.		
			1	2	3
Unsprayed ..	114	500	56	29	15
Bordeaux mixture	95	427	83	15	2
Lime sulphur ..	80	352	100	0	0

Under the conditions of 1926 the use of both lime sulphur and Bordeaux mixture resulted in a good control of the spotting on the canes, the measure of control given by two applications of lime sulphur (at 1 in 10 and 1 in 40 respectively) being consistently greater than that given by two applications of Bordeaux mixture at 12 : 12 : 100 and 6 : 6 : 100 respectively. Thus up till the middle of June the control by lime sulphur was perfect, and the use of Bordeaux mixture resulted in a decrease in the percentage of diseased canes from 44 on the unsprayed rows to 17. Later, new infection took place, and in November the percentage of healthy canes on the lime sulphur sprayed rows was 77, as against 39 on the Bordeaux mixture sprayed rows and 26 on the unsprayed. Even at this late stage it will be noticed that only 1 per cent. of the canes in the lime sulphur sprayed rows showed severe cankering whereas the percentage of such cases on the Bordeaux sprayed rows equalled that on the unsprayed rows, viz., 16.

Table IV. indicates that the use of lime sulphur and Bordeaux mixture not only resulted in a considerable increase in the average length of the canes, thus

TABLE III.

Summary of records made on November 15th, 1926, showing effect of 1926 spraying on the incidence of the disease.

Treatment.	No. of stools recorded.	No. of canes recorded.	Percentage of canes in disease categories.		
			1	2	3
Unsprayed	110	524	26	58	16
Bordeaux mixture	80	408	39	45	16
Lime sulphur ..	75	349	77	22	1

TABLE IV.

Summary of records made on May 7th, 1927, showing the effect of 1926 spraying on the vigour of the canes.

Treatment.	Total no. of canes recorded.	Total no. of buds examined.	Average no. of canes per stool.	Average length of canes.	Average length of cane per stool.	Average no. of buds per cane.	Percentage of dead canes.	Percentage of canes with dead tips.	Percentage of buds in development categories.				Average no. of Category 1 laterals per 100 ins. of cane.
									1	2	3	4	
Unsprayed	168	1,969	3.6	ins. 23.5	ins. 85	11.6	7.1	14.0	11	23	21	45	5
Bordeaux mixture ..	168	2,259	3.6	26.8	96	13.5	0.6	6.0	20	25	20	35	10
Lime sulphur ..	169	2,444	3.7	28.5	105	14.4	0.0	1.8	20	26	20	34	10

TABLE V.

The effect of spraying on weight of fruit crop. 1926 and 1928 experiments.

Treatment.	Weight of fruit crop. cwts. per acre. 1926. Year of Spraying.	1927.	1928. Year of Spraying.	1929.
Unsprayed	2.3	8.0	10.5	4.5
Bordeaux mixture ..	2.0	12.5	13.0	13.0
Lime sulphur	1.7	12.0	7.0	9.5

increasing the number of buds per cane, but it also resulted in an increase in the proportion of well developed laterals and a corresponding decrease in the proportion of dead buds, dead canes and canes with dead tips. For example, the average number of healthy vigorous laterals on a standard length of cane was doubled by the use of each of the spray fluids.

On the results set out in Table IV. there is little to choose between lime sulphur and Bordeaux mixture. This table also shows that a severe infection of Cane Spot results in a stunting of the cane and of the laterals and in the non-development of a large proportion of the buds, also in extreme cases in the killing of the tip portion of the cane or the killing of the entire cane.

Finally the reflection of the results set out in Table IV. is found in the weights of the fruit crop shown in Table V. It will be seen that the crop in 1927 yielded by the rows sprayed with lime sulphur and with Bordeaux mixture exceeds the crop from the unsprayed rows by about 50 per cent. of that crop. The slight difference between the crops from the rows sprayed with lime sulphur and Bordeaux mixture is not significant. Bearing in mind the fact that lime sulphur gave a better control of the spotting than Bordeaux mixture the question arises why the crop from the rows sprayed with the former did not exceed that yielded by the rows sprayed with the latter. It is suggested that direct injury to the cane resulting from the delayed dormant application of lime sulphur and indicated by the scorching of the foliage is one of the causes of this discrepancy.

3. *Spraying experiment in 1927.*

This experiment was carried out on a plot of five-year-old stools adjoining the plot sprayed in 1926.

Lime sulphur only was used in this experiment which was designed to determine:—

(i) The possibility of avoiding the scorching of the cane foliage recorded in 1926, by applying the " delayed dormant " spray at a slightly earlier stage in bud development ;

(ii) The comparative efficiency of " delayed dormant " applications at the strength used in 1926 (1 in 10) and at reduced strengths (1 in 15 and 1 in 20) ;

(iii) The comparative efficiency of the " delayed dormant " and " pre-blossom " applications used alone or in combination.

The " delayed dormant " application was made on March 28th, when the most advanced buds on the canes had pushed out *not more than ½ inch* (Plate V.) (c.f. stage of application in 1926) ; the pre-blossom application was made on May 18th, when the canes had reached the same stage of development as that adopted in the 1926 experiment. The " delayed dormant " application was made at the strength of 1 in 10, 1 in 15 and 1 in 20. For the pre-blossom application the strength adopted in 1926 (1 in 40) was used throughout. The plot consisted of eleven rows, each approximately 180 feet long. Four rows were not sprayed and acted as controls to the experiment, one row was sprayed with the pre-blossom spray (at 1 in 40) alone ; one half of each of the remaining rows received the " delayed dormant " and the pre-blossom applications of the spray, and the other half received the " delayed dormant " application only. On two rows the strength of the " delayed dormant " spray was 1 in 10, on two rows 1 in 15, and on the remaining two rows 1 in 20.

After the application of the " delayed dormant " spray the plantation was examined daily with the object of recording any scorching of the foliage due to the spraying ; no such scorching was detected. One month after spraying (on April 28th) a detailed examination was made of the canes of every tenth stool on each of four rows receiving the four main treatments. Each bud on these canes was examined and classified according to its development. The method used was a slight modification of that used in the same year for determining the vigour of the cane sprayed in 1926 (see p. 82). The object was to determine any possible direct effect of the " delayed dormant " spray on the development of the canes. These records showed that there was at this stage no significant difference between the development of the sprayed and unsprayed canes. The first incipient infection spots on the unsprayed canes were recorded on May 24th, but at this time they were very scarce and difficult to detect, indeed it was not till August that infections were seen in quantity.

No detailed records of cane development were made in 1928, but it will be seen from Table VI. that the average weight of the fruit crop yielded by the unsprayed rows in 1928 did not differ significantly from that yielded by the sprayed rows.*

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* There is a possibility that the slight increase in the weight of the crop from the sprayed rows over that yielded by these rows in 1927 as shown in Table VI. may be due to the spraying.

Under the seasonal conditions, therefore, of 1927 the effect of the attack of the disease on the canes was very slight.

Detailed records of the incidence of the disease made in September, 1927, on all the sprayed and unsprayed rows, and summarized in Table VI., indicated that in 1927 the control obtained by each of the treatments was equally good and resulted in raising the percentage of normal (category 1) canes from 40 to approximately 70 and in reducing the percentage of canes showing concentrated "superficial" spots from 42 to approximately 20. There are indications, however, that the use of the pre-blossom spray alone or both the sprays in combination gave a better control of "superficial" spotting than the use of the "delayed dormant" spray alone.

TABLE VI.

Summary of records made on September 19th, 1927, showing the effect of spraying with lime sulphur in 1927, on the incidence of disease in that year.

Treatment.	Total stools recorded.	Total canes recorded.	Percentage of canes in disease categories.			Percentage of canes with "superficial" spots.	Crop weight, cwts. per acre.	
			1	2	3		1927.	1928.
Unsprayed	237	1,331	40	48	12	42	15.4	13.3
Two applications; at 1 : 10 and 1 : 40	61	342	70	28	2	22	13.0	14.7
One application; at 1 : 10	59	350	74	22	4	23		
Two applications; at 1 : 15 and 1 : 40	61	359	73	24	3	18	13.6	17.0
One application; at 1 : 15	58	340	69	29	2	25		
Two applications; at 1 : 20 and 1 : 40	59	269	70	26	4	13	11.5	13.8
One application; at 1 : 20	61	321	66	30	4	23		
One (pre-blossom) application at 1 : 40	61	278	66	32	3	13	—	—

The following conclusions may be drawn from the above results:—

(i) The main efficacy of the spray fluid consists in the direct reaction of that fluid on the fungal fructifications on the "superficial spots" of the fruiting canes, rather than in the deposition of a protective layer of fungicide on the surface of the new canes, because the spray was equally effective whether applied before or after the appearance of the new canes above soil level. That the action of the spray is direct and not protective is further supported by the observation made on numerous occasions that the spray fluids (without a "spreader") spread very readily and evenly over the surface of the fruiting canes whereas they do not spread readily on the waxy surface of the new canes but tend to gather into drops.

(ii) It may further be concluded that the initial main infection of the canes in 1927 occurred after the application of pre-blossom spray, otherwise the control yielded by that application would not have been so satisfactory as that yielded by the "delayed dormant" application.

These results and conclusions will be discussed when the concluding experiment of the series (that of 1928) has been described.

4. *Spraying experiment in 1928.*

This experiment consisted of a further spraying (after a year's interval) of the plot sprayed in 1926 with lime sulphur and Bordeaux mixture according to the schedule used in that year, but with the following modifications:—

(1) "spreaders" were not used and

(2) the stage of application of the "delayed dormant" was the earlier one adopted in the 1927 experiment (Plate V.).

The "delayed dormant" application was made on March 22nd and the pre-blossom application on May 29th.

Observations made after spraying showed that no leaf scorching occurred as a result of the application of either of the sprays.

The results of this experiment which are the cumulative results of two seasons' spraying (in 1926 and 1928) will now be summarized. The records of the incidence of this disease on the canes and of the vigour of growth of the stools were made at the same time, i.e., at the beginning of February, 1929. The procedure was as follows. The canes of each recorded stool were first examined and classified according to the intensity of the spotting; the stool was then thinned, i.e., all very dwarf and useless canes were cut out, and the remaining canes were tipped and their length then measured. These records are summarized in Tables VII. and VIII. and the weight of the fruit crop from the sprayed and unsprayed rows in 1928 and 1929 are given in Table V. The main conclusions of interest are as follows:—

(i) The control of the disease by Bordeaux mixture was increased as a result of the second season's spraying, and was approximately equal to that given by the lime sulphur; by the end of 1928 the disease on the rows sprayed by both these fungicides had been very nearly eliminated.

(ii) The vigour of growth of the cane sprayed with Bordeaux mixture, and the corresponding cropping power of the stools had been steadily maintained (and probably slightly increased).

(iii) On the other hand the vigour of growth and cropping power of the rows sprayed with lime sulphur had markedly decreased in spite of the effective control of the disease by this fungicide. The weight of the crop from these rows in 1928 fell below that yielded by the unsprayed rows, and the vigour of the lime sulphur sprayed canes in 1928 was only equal to that of the unsprayed canes in 1926. At the same time the weight of the crop from the unsprayed rows, although it rose a little in 1928, fell very rapidly in 1929 corresponding to a marked fall in vigour of growth of the cane in 1928.

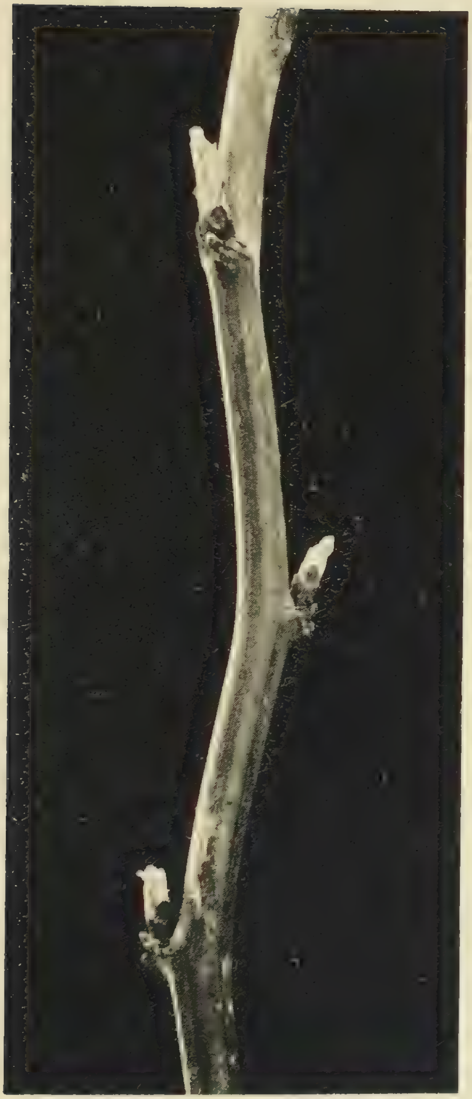
TABLE VII.

Summary of records taken in February, 1929, showing the effect of spraying carried out in 1926 and 1928 on the incidence of the disease.

Treatment.	Total stools recorded.	Total canes recorded.	Percentage of canes in disease categories.			Percentage of canes with "superficial" spots.
			1	2	3	
Unsprayed	174	732	46	26	28	68
Bordeaux mixture ..	124	655	97	3	0	14
Lime sulphur	123	616	98	2	0	18

It is concluded from these facts that the application of lime sulphur in 1928 resulted in direct injury to the fruiting cane (reflected in the fall of the 1928 crop), and to the new canes, as shown by the decrease in vigour of that cane in 1928 and the diminished crop of 1929. As the new cane of 1928 was injured it follows that a part, at any rate, of the damage was caused by the "pre-blossom" application in 1928 (at 1 in 40). Unfortunately, as the whole of the sprayed rows received both applications of the spray, it is not possible to determine whether the damage was, in addition, partly due to the delayed dormant application and if so the degree of damage caused by such application.

An additional factor which accentuated the damage due to the incidence of the disease and to the lime sulphur respectively was the severe frosts in the latter part of the winter of 1928-29. When the records of the vigour of the stools were made (in February, 1929) the canes were still dormant. As the season advanced and the buds commenced to grow out it was noticed that the buds on



The correct stage at which to make the delayed-dormant application of the spray (var. *Baumforth Seedling B*). Natural size.

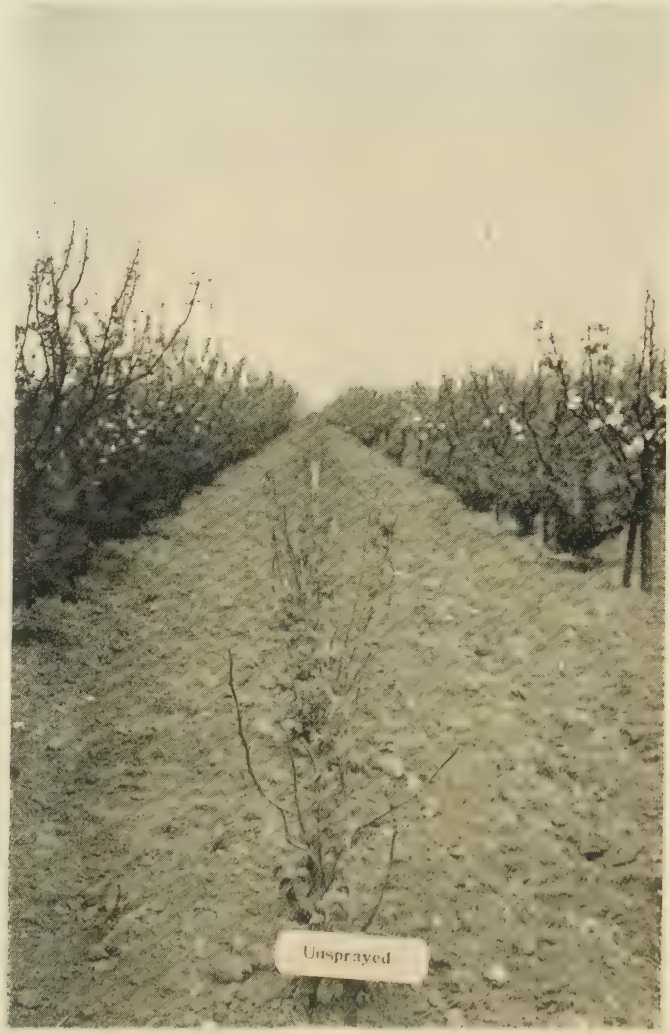


FIG. 1.

An unsprayed row in the 1926 and 1928 spraying experiment on May 16th, 1929, showing the effect of the disease and the severe

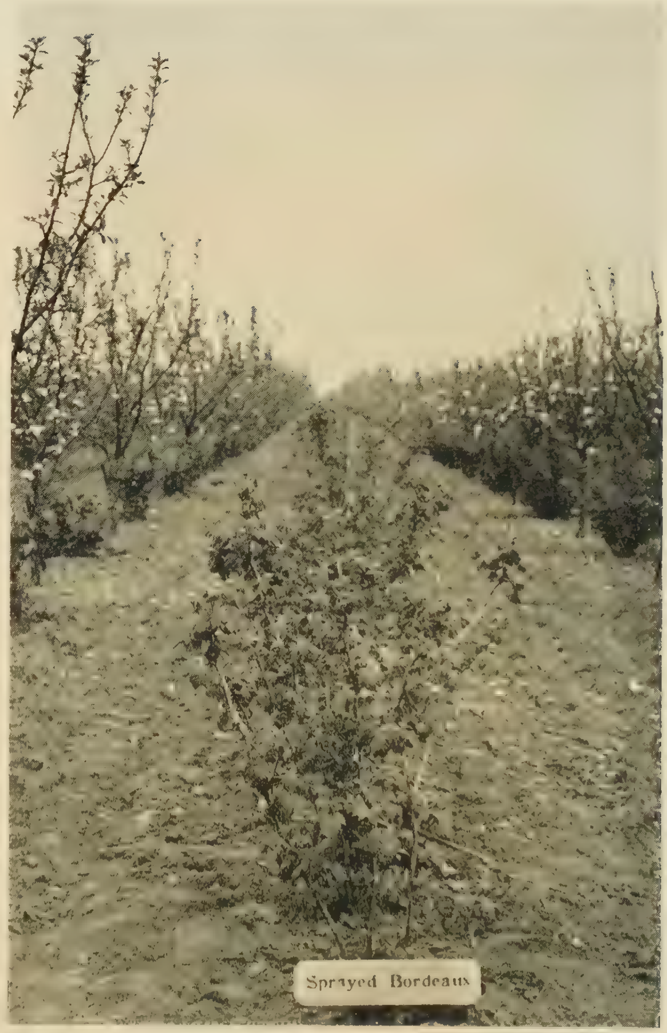

Sprayed Bordeaux

FIG. 2.

A row in the 1926 and 1928 experiment sprayed with Bordeaux Mixture. Photographed on the same day as Fig. 1.

the apical portions of many of the canes in the unsprayed rows and in the rows sprayed with lime sulphur were not developing and that many entire canes in these rows were still in the dormant condition with the exception of one or two of the basal buds. On cutting such canes it was found that the tissues of the affected portions of the canes were in process of turning yellow, the discoloration starting from the tip and extending downwards. This phenomenon was most severe on the unsprayed rows but was also very serious on the lime sulphur sprayed rows. Very few canes in the Bordeaux mixture sprayed rows were effected, and in the early part of May, 1929, these rows appeared green and normal, whereas those sprayed with lime sulphur and the unsprayed rows were very barren and poorly furnished with laterals. The striking difference in the appearance of the rows at this time is shown by Plate VI., Figs. 1 and 2. It is therefore evident that the figures in Table VIII. give an exaggerated idea

TABLE VIII.

Summary of records made in February, 1929, showing the effect of spraying carried out in 1926 and 1928 on the vigour of the stools.

Treatment.	Average No. of canes per stool *before thinning*.	Average No. of canes per stool *after thinning*.	Percentage of canes cut away.	Average length of canes *after thinning*	Average length of cane *per stool* after thinning.
				ins.	ins.
Unsprayed	4.1	2.7	35	21.4	58
Bordeaux mixture ..	5.6	4.0	29	26.4	104
Lime sulphur ..	5.4	3.4	36	23.7	82

of the vigour of the stools on the unsprayed rows and the lime sulphur sprayed rows, as they do not allow for the cane which subsequently died as a result of the severe frosts. It is further evident that the susceptibility of the canes to frost injury was in part due to the severe Cane Spot infection and also to the direct effect of the lime sulphur on the canes and that this susceptibility was minimised by the use of Bordeaux mixture. Later in the season, as the new canes grew up, the differences in vigour between the rows subjected to the different treatments were not so apparent for the reason that during the season of 1929 the attack of Cane Spot did not appreciably affect the vigour of growth of the new cane. At the end of the season no difference in vigour between the rows sprayed with lime sulphur and Bordeaux mixture and the unsprayed rows, was noticeable to the eye. Although no records on the vigour of the stools during this season were taken it is safe to say from observation that at the end of 1929 the stools sprayed with lime sulphur in 1928 had to a great extent recovered their vigour.

5. *A discussion of the seasonal fluctuations of the disease in relation to routine spraying.*

In this discussion the plot of stools sprayed in 1926 and 1928 will be referred to as spraying plot No. 1, and that (one year older) sprayed in 1927 only, as spraying plot No. 2. Unless mention is made to the contrary the notes on the seasonal severity of the attack refer to the variety *Baumforth B.*

A consideration of the data collected in the four years (1926-29) during which the spraying experiments were in progress, makes it evident that the severity of the attack of the disease varies very greatly from season to season. This fluctuation of the disease is clearly correlated with fluctuations in the weather from season to season.

The infection of the canes by the fungus depends (i) on the maturing of the ascocarps, (ii) the discharge of the ascospores and the distribution of conidia and (iii) the successful infection of the canes by these discharged spores. Evidence is forthcoming that each of these factors is modified by variations in external weather conditions. Observations made during the seasons 1925 to 1929 show that the earliest date in the spring at which mature ascocarps are to be found in quantity varies greatly from season to season. Mature ascocarps were found in large numbers in the early part of May in 1925 and from the middle of April onwards in 1926. In 1927, on the other hand, no mature ascocarps were found till May 17th, and at this time they were not present in large numbers but were only discovered on occasional canes after searching carefully. In 1928 they were not recorded till April 24th and at this time were very scarce. After this date they matured very rapidly and by May 24th many were in a discharged condition. In 1929 it was only with great difficulty that mature ascocarps were found at any time in the season.

With regard to the influence of environment on the discharge of ascospores and the abstriction of conidia, it has been found that the discharge of ascospores is accelerated by placing the ascocarps in a saturated atmosphere and that the abstriction of conidia is brought about by changes in the humidity of the environment (p. 77). That the optimum conditions for infection are of a specialized nature is also evident from the results of the inoculation experiments carried out.

The amount of damage caused to the cane by the disease depends, in addition to the concentration of the spot lesions, on the stage in the development of the young canes at which the main infections take place, and also on the weather conditions to which the canes are subject after infection. Thus, the damage caused by early infections when the cane is young and tender is much greater than that caused by infections taking place later in the season. Observations have shown that the time at which the initial main infection takes place differs considerably from season to season. Thus in 1926 incipient infection spots were abundant on May 26th ; in 1927 a very few incipient infections were recorded on May 24th, but the main mass of infection spots did not appear till late in June ; in 1928 young spots were abundant early in June, but at no period in the season of 1929 were young spots abundant. In none of the seasons in which the disease has been observed by the writer have incipient infection spots been noticed earlier than May 24th. Unfortunately the inoculation experiments carried out yielded no information as to the time elapsing between the infection and the appearance of the spots. Jones working in America on the Black Raspberry gives an incubation period of from six to nine days for infection by conidia (21). If the incubation period in this country is similar it would appear that the earliest " critical month " for infection is May. As mature ascospores have been observed as early as the middle of April (at which time the canes are about 6 inches high), it is possible that infection may take place in this month. Infections may occur in quantity therefore from May onwards. It is unlikely, however, that infections occurring later than July will damage the canes to a great extent, for by this time the main growth of the cane has taken place. Infections at the end of August onwards result in the formation of the superficial type of spot. The " critical months " of infection would therefore appear to be April (possibly) and May, June and July, and in attempting to correlate cane infection with weather conditions the weather during these four months has been particularly noted.

With regard to the comparative importance of the ascigerous and the conidial stage of the fungus in the spread of infection, it may be said that during the critical months of infection referred to above, conidia have been found from time to time associated with acervuli, but their appearance is very sporadic and only on one or two occasions have they been found to be present in any great quantity. On the other hand, the production and discharge of ascospores in large quantities has been observed to take place with great regularity during the critical months of every year in which observations have been made. Further, the walls of the conidia are mucilaginous and the conidia when abstricted tend to remain on or around the acervulus until they are dispersed by rain ; the ascospore on the other hand is discharged forcibly into the air and is carried by the wind. In the majority of cases the spore is carried to canes in the immediate vicinity of the ascocarp (i.e., to canes belonging to the same stool), but in analysing figures on the intensity of the spotting on the sprayed plots, evidence has been found that infection may take place at a considerable distance from the source of the infection, i.e., on stools in a neighbouring row or further afield. It is therefore suggested that the main source of the early or primary infection of the young canes is the ascocarp. Later as has been already observed, conidia are formed on the young spots and are probably washed to other parts of the same cane and produce secondary infection spots.

Of the six seasons during which the disease has been under observation the attack in 1924 was outstandingly severe, those in the seasons of 1925, 1926 and 1928 very severe, and in the seasons of 1927 and 1929 very slight. Unfortunately no numerical data are available with which to estimate the attack in 1924, for no detailed observations were made on the development of the fungus on the fruiting canes in that year, when little was known about the disease. However the cankering, spotting and distortion of the canes of *Baumforth B.* during that year was the most serious yet observed at the Research Station and the crop yielded in 1925 was, as before mentioned, very small. It is suggested that the seriousness of the attack was due in part at least, to the fact that the rainfall during the "critical months" of the year, i.e., April to July, was excessively heavy. The weather conditions in 1925, during the critical months of infection, were also ideal for the spread of disease, with the exception of June, which was a very dry month. The serious infection of the canes during this year probably accounts for the fact that the average crop on the nine rows of spraying plot No. 2 fell from 6.5 cwts. per acre in 1925 to 4.4 cwts. in 1926, instead of increasing as would normally happen in a young plantation in its third year. In 1926 conditions were again very favourable for an early infection of the canes. The ascocarps matured in large numbers at a very early date ; April and June were excessively wet months and May and July moderately wet, the rainfall in each of the four months being well distributed throughout the month.

The very slight attacks of the disease in 1927, as seen from the very slight effect of the spraying on the crop of the following year, is probably due to two main causes, (1) the lateness of the initial main infection of the canes in 1927, and (2) the very wet character of the season from early June onwards. The lateness of the initial main infection was probably due to the late maturing of the ascocarps already mentioned and the exceptionally dry weather of April and May ; infection as a result did not get under way till late in June.

That the attack of spotting was less intense in this year than in 1926 or 1928 is shown by comparing Tables III., VI. and VII., when it is seen that in 1927 the percentage of seriously infected (category 3) canes was only 12, as compared with 16 per cent. in 1926 and 28 per cent. in 1928. The effect of the impairment of the water conducting system of the canes by the lesions when formed was probably minimized by the continuously wet and cold weather from June onwards. These conditions so unfavourable for the growth of the young canes may also account for the fact that the crop of 1928, although unaffected by the spraying, was not greater than the 1927 crop.

The seriousness of the 1928 attack is readily seen from the very pronounced fall of the weight of the 1929 crop from unsprayed rows on Plot No. 1, and in the fall in the vigour of the canes on these rows in 1928, as shown in Tables V.

and VIII. That this is in part directly due to the effect of the lesions on the cane is evident from the fact that 28 per cent. of the canes of this year were graded in disease category 3. An additional cause was, as already pointed out, the severe frosts in the early months of 1929. The weather conditions during April, May and June in 1928 were favourable to the spread of infection. June in particular was a very wet month and it was during the early part of this month that the first main appearance of young spots was noticed.

Finally 1929 was a year of extremely slight attack. Mature ascocarps were very rarely observed during this season, and incipient infection spots were not numerous at any time. The unsprayed rows on Plot No. 1, so badly impaired by the disease and frost in 1928, grew up vigorously in 1929, and by the end of the season these rows were indistinguishable from the Bordeaux sprayed rows. The slight character of the attack in this year is clearly related to the fact that April, May, June and July were exceptionally dry months.

It will be seen from the above observations that in four of the six seasons dealt with, the attack of the disease has been a serious one for reasons which have been discussed. Of these four seasons, three (1924, 25 and 26) were consecutive seasons. In view of this fact it is suggested that if possible spraying should be carried out annually. Although the successive sprayings carried out in the alternate seasons of 1926 and 1928 gave a good cumulative control, which was maintained in 1927, it must be remembered that the attack of the disease in 1927 was very slight. Further, it was found that the crop from the rows sprayed with lime sulphur in 1927 (without direct injury) was, in common with the unsprayed rows on the same plot, considerably reduced as a result of the severe attack in 1928, showing that the effect of the spraying in 1927 had not persisted in 1928.

D. SUGGESTED ROUTINE CONTROL SCHEDULE.

1. Badly cankered and spotted canes should as far as possible be cut out and burnt during the spring thinning of the stools. In seriously infected plantations this need not be attempted till after the first year's spraying.

2. The fruiting canes should be sprayed annually according to the following programme:—

(a) *Times of application.*

(i) *Delayed-dormant stage*—When the cane buds have pushed out *not more than ½ inch* (see Plate V).

(ii) *Pre-blossom stage*—When the first flower buds are just showing white tips.

(b) *Strengths of sprays.*

(i) *Delayed-dormant application.*
Bordeaux mixture 12 : 12 : 100
or Lime sulphur 1 in 15.

(ii) *Pre-blossom application.*
Bordeaux mixture 6 : 6 : 100
or Lime sulphur 1 in 40.

From the results of the experiments described above, it is clear that Bordeaux mixture is the safest and most reliable spray to use. Many growers, however, prefer to use lime sulphur owing to the greater ease with which it is prepared, and because it is less likely to cause an obstruction of the lance nozzles ; for this reason lime sulphur has been added as an alternative in the above programme. However, as it was found that under certain seasonal conditions two applications of lime sulphur at 1 in 10 and 1 in 40 may cause direct injury to the cane, the suggested strength of the delayed-dormant application has been reduced to 1 in 15. In 1927 an equally good control was obtained by using delayed-dormant sprays at 1 in 10, 1 in 15, or 1 in 20, but in this year the disease attack was not serious, so that it is possible that in years of serious infection the effectiveness of the 1 in 15 wash may not be as great as that at the 1 in 10 strength. In the same year an equally good control was obtained on rows which received only a single spraying at the delayed-dormant stage or at the pre-blossom stage. After a badly infected plantation therefore has been cleaned up by one year's spraying with the full programme, the risk of direct lime sulphur injury to the cane could be further reduced by omitting the pre-blossom application, a procedure that would also appeal to growers who find it impossible to spray their raspberries twice annually. The omission of the delayed-dormant application is not to be recommended, for there is a risk that in certain seasons considerable infection might take place before the pre-blossom stage was reached. Further, as it is only necessary to deposit a film of the spray on the surface of the fruiting canes, there is obviously a great advantage in applying the spray before the laterals grow out or the young canes begin to grow up round the fruiting canes.

Finally it must be emphasized that the best results will be obtained by a regular annual spraying. Even if the effects of the disease are eliminated by spraying for a year or two, the production of "superficial" infection spots at the end of the season will not entirely cease, and at the end of any spraying year there will be enough fungal fructifications on the canes to bring about a serious attack of the disease the following year if the conditions are favourable for infection and the spraying is allowed to lapse. One regular spraying every year is therefore to be preferred to an occasional spraying according to the full programme.

SUMMARY.

1. The symptoms of the *Cane Spot* disease as they occur in Great Britain on canes of the red raspberry (*Rubus idæus L.*) are described.

2. The isolation of the pathogen in pure culture, inoculations to establish its pathogenicity, and salient points in its seasonal cycle on raspberry canes are summarized. It is found to conform closely to descriptions of *Plectodiscella veneta* (Speg.) Burk. the cause of the *Anthracnose* disease of *Rubus* sp. in America.

3. Certain commercial varieties of raspberry are found to be more susceptible to the disease than others. A list of extremely susceptible varieties is given.

4. Experiments are described which show that the disease on highly susceptible varieties may be controlled by a modification of cultural methods, and by spraying with Bordeaux mixture or with lime sulphur.

5. The severity of attack is shown to vary greatly from season to season. The causes of this variation are discussed and a routine schedule of control measures is suggested.

ACKNOWLEDGMENTS.

In conclusion, the writer wishes to express his particular indebtedness to Dr. H. Wormald and Mr. Norman Grubb for unstinted advice and guidance throughout, and to Miss A. M. Frampton for assistance in the cultural work and field recording.

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A PRELIMINARY ACCOUNT OF CHROMOSOME BEHAVIOUR IN THE POMOIDEÆ.

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In an earlier paper (Darlington and Moffett, 1930 (6)) the theoretical conclusions of chromosome behaviour in cultivated apples were considered in detail, and the practical inferences of these investigations were also described exhaustively by Crane and Lawrence (1930) (3). Since these papers appeared I have extended my studies to the other groups of the Pomoideæ, and a complete account of these studies will be published elsewhere, but certain of the results are of direct interest to the pomologist, and in the present paper an attempt is made, as far as the technicalities of the subject will allow, to give a simple account of chromosome behaviour, and the practical aspects of the investigations.

Every plant is built up of cells which have all been derived from a single cell by repeated division. This single cell contains a deeply staining body—the nucleus, which as the cell is preparing for division differentiates into a number of rod-like bodies which are called chromosomes. The number of these chromosomes is definite for each species, and very often the numbers and shapes of the chromosomes are constant for the genus. The chromosome complement of a given plant normally consists of two sets, one set being derived from each parent. Growth proceeds by the division of cells. At the division of somatic or vegetative cells each chromosome splits longitudinally and half passes to each daughter cell, so that in consequence the two new cells are an exact repetition of the old. This equal division is important because on the chromosomes are situated the factors or genes which determine hereditary characters. Because of this even division of chromosomes at somatic divisions, all individuals produced by vegetative propagation are normally identical with the original.

Since sexual reproduction takes place by the fusion of two cells, it is obvious that unless there is some mechanism by which the chromosome number is reduced, each succeeding generation will have doubled the chromosome complement. The reducing mechanism is supplied by meiosis, the type of cell division which precedes the formation of reproductive cells. At this division the chromosome number is halved. The chromosomes within each set are differentiated from each other, but have corresponding or homologous chromosomes within the other set. Thus the chromosome complement of a diploid plant (i.e., plant having two complete sets) may be represented as AA, BB, CC, DD, etc. During the formation of sex cells and gametes the chromosomes come together in pairs, A with A and B with B, so that instead of having 2n single chromosomes, we have n paired or bivalent chromosomes. Only homologous chromosomes can pair, so that normally pairing only takes place between the two sets and not within them. The paired chromosomes then separate again, giving rise to daughter cells with n instead of 2n chromosomes, i.e., A, B, C instead of AA, BB, CC, etc. This process takes place during the formation of pollen and ovules, the resulting pollen grains and egg cells only containing half as many chromosomes as the somatic cells.

During the process of fertilisation the nuclei containing the halved or haploid chromosome numbers from each parent come together and thus restore the original number in the new cell from which the seed and eventually the new plant originates.

The pairing of chromosomes at meiosis and their ultimate separation determines the segregation of hereditary characters. For example—if a red flower is crossed with a white flower and red is dominant, all the F_1 progeny will be red. The red factor is carried on one chromosome and the white on the corresponding chromosome from the other parent. Thus each cell of the F_1 plant contains a red and a white factor on different chromosomes, the white being rendered invisible by the dominance of the red. At meiosis, however, the two chromosomes pair and then disjoin, one passing to one side of the division and the other to the other side, so that the red and white factors become separated again. This process takes place in pollen and ovules, giving 50 per cent. of each carrying the white factor and 50 per cent. the red. On selfing or back crossing two reproductive cells carrying the white may come together again and the white form be recovered.

The process of reduction in germ-cell formation is often complicated among our cultivated fruits owing to their hybrid origin and complex chromosome constitution. Instead of possessing two homologous or corresponding sets of chromosomes, many plants are polyploids—that is they have more than two sets of chromosomes. Those with an even number of sets such as tetraploids with four and hexaploids with six are usually more productive than those having an odd number, triploids with three or pentaploids with five. In polyploids there are three or more corresponding chromosomes of each type, so that instead of forming just pairs of chromosomes, associations of three or more chromosomes may be formed. It is obvious that if these associations have an odd number of chromosomes in them they cannot divide evenly, and consequently irregularities and sterility will inevitably follow. Examples of reduced fertility due to an odd-multiple polyploid constitution has been abundantly shown in Rubus (2) and Prunus (4). Apart from the effect on fertility produced by chromosome aberrations, polyploidy has an important effect on incompatibility and the genetical behaviour of a plant. It has been shown that incompatibility is determined by genetic factors just as are morphological characters, and since the segregation of genetical factors is determined by the pairing and division of chromosomes at reduction, it is obvious that more chromosomes of each type will considerably complicate the segregation. Crane and Lawrence have shown that in a polyploid plant exhibiting incompatibility, the gametes will carry more than one factor for incompatibility, and the possibility of the occurrence of interactions favourable to a greater variation in pollen tube growth must be considered. Consequently varying degrees of incompatibility might be expected in a polyploid.

It is necessary therefore to know whether a plant used for breeding purposes is a diploid or of a more complex chromosome constitution.

In a recent paper (6) the hypothesis was put forward that the thirty-four chromosomes of the diploid apple represented a more complex complement than that of a normal diploid. From the chromosome morphology and pairing it was concluded that the primary basic number in Pyrus is seven, and that the thirty-four chromosomes of the diploid are of seven types, three of which are represented six times and four of which are represented four times. In other words the supposedly diploid apple is a complex polyploid—a tetraploid in respect to four chromosomes and a hexaploid in respect to six—and its breeding behaviour will be that of a polyploid.

Since such a hypothesis, depending as it does chiefly upon the observations of chromosome pairing and morphology, is necessarily difficult to prove, the present investigations were undertaken to see how far the evidence deduced from other groups of the Pomoideæ supported the hypothesis. Such a change in chromosome constitution when it occurs under experimental conditions is usually accompanied by a distinct change in the morphological characters of the plant ; and it seemed possible that the Pomoideæ as a group might owe their special morphological characters to such a rearrangement of their chromosome complement. On these grounds it was predicted that a basic number of seventeen would be found throughout the group, even though other workers had reported different chromosome numbers.

Full details of the methods used, together with a discussion of the cytology, will be found elsewhere (9).

TABLE I.

Genera of the Pomoideæ (after Engler and Prant (7)) *with their chromosome numbers.*

	Chromosome numbers.	
	P.M.C.	Somatic.
1. *Cotoneaster* Medic.		
Section I. *Eucotoneaster* Focke.		
Cotoneaster procumbens	—	34
congesta Baker	51/2	51
rotundifolia Wall.	—	68
microphylla Wall.	—	68
vulgaris Lindl.	34	—
bullata Bois.	34	—

	Chromosome numbers. P.M.C.	Chromosome numbers. Somatic.
Section II. *Pyracantha* Rom.		
Pyracantha Gibbsii A. B. Jackson	—	34
coccinea Lalandii Dipp.	17	—
2. (*Nagelia* Lindl.)		
3. *Osteomeles* Lindl.		
Osteomeles Schwerinae Schneid	17	34
4. *Cydonia*.		
Section I. *Eucydonia* Focke.		
Cydonia vulgaris Pers.	17	34
Section II. *Chaenomeles* Lindl.		
Cydonia (Chaenomeles)		
japonica Pers.	17	34
cathayensis Hemel.	17	—
Maulei T. Moore	17	—
5. (*Docynia* Decsne.)		
6. *Pyrus* Tourn.		
Sub-genus (I.). *Pirophorus* Medic.		
Pyrus communis L.	—	34
vars. "Fertility"	17	34
"William's Bon Chretien"	17	—
"Beurré Giffard"	17	—
Sub-genus (II.). *Malus* Tourn.		
Pyrus Malus L.		
vars. "Rev. W. Wilks"	17	—
"Baumann's Reinette"	17	—
"Washington"	51/2	—
Sub-genus (III.). *Hahnia* Medic.		
Pyrus torminalis Erhr.	17	—
Sub-genus (IV.). *Sorbus* L.		
Pyrus Sorbus Gaertn.	17	-
Pyrus minima Hedl.	51/2	—
Pyrus aucuparia L.	—	34
Pyrus Aria L.	17	
Sub-genus (V.). *Aronia* Pers.		
Pyrus arbutifolia L.	34	68
Pyrus hybrida Moench.	17	-
7. *Raphiolepis* Lindl.		
Raphiolepis Delacourii Andre	17	34
Raphiolepis indica Lindl.	17	
8. *Eriobotrya* Lindl.		
Eriobotrya japonica Lindl.		34
9. (*Photinia* Lindl.)		
Section I. *Euphotinia* Focke.		
Section II. *Heteromeles* Rom.		
10. *Pourthiaea* Decsne.		
Photinia (*Pourthiaea*) *villosa* DC.	34	68

	Chromosome numbers.	
	P.M.C.	Somatic.
11. *Amelanchier* Medic.		
Amelanchier stolonifera Wiegand	—	68
laevis Wiegand	34	68
rotundifolia Roem.	—	68
canadensis Torr. and Gray	34	68
12. *Stranvaesia* Lindl.		
Stranvaesia Davidiana undulata Decsne.	17	34
13. *Mespilus* L. (including *Crataegus*).		
Mespilus germanica L.	17	—
—*Crataegus*		
Crataegus monogyna Jacq.	17	34
oxyacantha L.	17	34
stipulosa Stend.	—	34
chlorosarca Maxim.	17	—
monogyna var. cabulica. Hort.	51/2	—
rotundifolia Moench.	—	68
cognata Sarg.	34	—
pruinosa Koch.	34	—
apposita Sarg.	34	—
pedicillata Sarg.	34	—
14. (*Chaenomeles* Lindl.)		

The genera in brackets have not been examined.

The table shows that the basic number of 17 runs throughout the Pomoideæ and that diploids, triploid and tetraploids occur, all the species and varieties being orthoploid (i.e., having a chromosome number which is an exact multiple of the basic number). Aneuploid forms (with a chromosome number not an exact multiple of the basic number) have only been found in the progeny of triploids, and these are in general of weak constitution and are probably incapable of existing under natural conditions.

In a recent paper Crane and Lawrence (3) have shown that out of the ninety-one apple varieties that have been examined cytologically, twenty-seven are triploids. Thus it is shown that triploidy is of wide occurrence in cultivated apples. Although there is a considerable variation in the proportion of good pollen in both groups, the diploids and triploids are clearly distinguishable, the triploids having a maximum pollen germination percentage of 30 as against the minimum of 50 in the diploids.

The chromosomes are all small, the largest being about 3μ long and the smallest about 1μ. One type—the largest, a subterminally constricted chromosome—is represented four times in the diploid, six times in the triploid and eight times in the tetraploid (Figs. 1-5).

Preparations of pollen mother-cells afforded the chief material examined, supplemented where possible by root tip preparations.

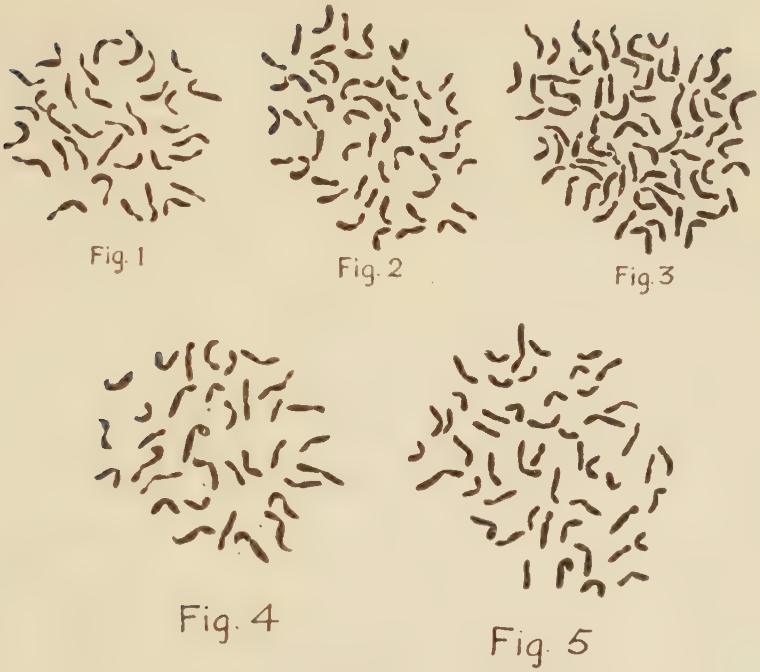

Fig. 1 Fig. 2 Fig. 3

Fig. 4 Fig. 5

TEXT FIGURES 1-5: SOMATIC DIVISIONS FROM ROOT TIPS. x4000.

(1) Crataegus oxyacantha $2n=34$.
(2) Cotoneaster congesta $2n=51$.
(3) Crataegus rotundifolia $2n=68$.
(4) Apple "Lane's Prince Albert," $2n=34$.
(5) Apple "Genet Moyle," $2n=51$.

POLLEN MOTHER-CELL DIVISIONS.

Throughout the diploid forms the chromosomes at the first metaphase show secondary pairing similar to that found in *Pyrus* (6). Seventeen bivalents are usually formed by pairing at prophase, as in normal diploids, but at some stage after prophase the pairs of bivalents come to lie together in groups of four or six chromosomes each, giving rise to the phenomenon of "secondary pairing." The extent of this pairing varies, but never less than seven groups have been observed and never more than six chromosomes (3 bivalents) in a group (Figs. 6-8). The maximum association observed was three groups of six chromosomes and four groups of four chromosomes in the apple variety Northern Spy (Fig. 7), the seventeen pairs of chromosomes appearing as seven groups. Secondary pairing is considered to indicate an affinity between the chromosomes, and this is supported by the fact that quadrivalents and sexivalents do occur occasionally in the so-called diploids, while corresponding higher associations of chromosomes are found in the "triploids" and "tetraploids."

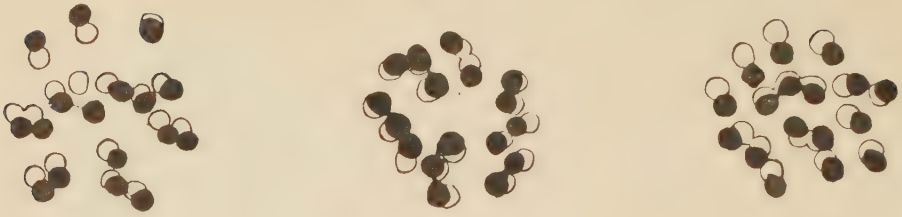

Fig. 6 Fig. 7 Fig. 8

TEXT FIGURES 6-8: POLAR VIEWS OF METAPHASE OF THE FIRST DIVISION. x5000.
(6) Cydonia cathayensis, $2n=34$.
(7) Apple " Northern Spy," $2n=34$.
(8) Raphiolepis indica, $2n=34$.

In triploid forms, the proportion of multivalents is higher than in diploids or tetraploids (Figs. 9-10). This is probably due to the conditions of chromosome pairing at prophase in triploids, and will be discussed more fully elsewhere (9).

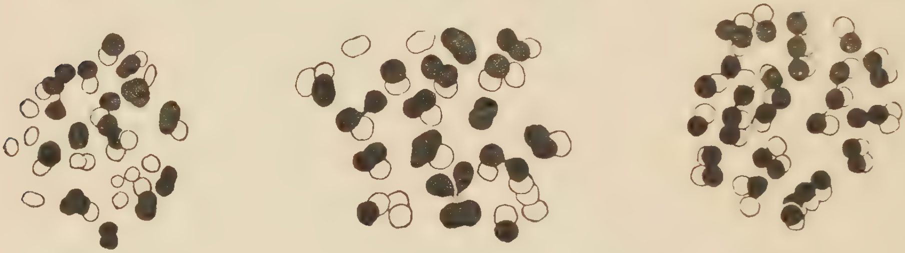

Fig. 9 Fig. 10 Fig. 11

TEXT FIGURES 9-11: POLAR VIEWS OF METAPHASE OF THE FIRST DIVISION. x5000.
(9) Cotoneaster congesta, $2n=51$.
(10) Crataegus monogyna var. cabulica, $2n=51$.
(11) Amelanchier canadensis, $2n=68$.

Tetraploid species at first metaphase show secondary pairing of a type similar to that found in diploids (Fig. 11). On account of the greater number of chromosomes, however, it is more difficult to distinguish between true association of chromosomes and secondary pairing.

CHROMOSOME NUMBERS IN THE PROGENY OF TRIPLOIDS.

Chromosome numbers were determined in nineteen seedlings of crosses between diploid and triploid apples and of twelve seedlings of triploids crossed by triploids and triploids selfed. The seedlings were raised by Messrs. M. B. Crane and W. J. C. Lawrence of this Institution.

It will be noticed that seedlings with forty-one chromosomes are in excess of those with any other number. This is unusual: in the triploids of other Orders previously observed, either the highest number of gametes have a chromosome

TABLE II.

Chromosome Numbers of Seedlings from Crosses between Diploids and Triploids.

	No. of Chromosomes										
	37	38	39	40	41	42	43	44	45	46	47
Lane's Prince Albert (2n) × Bramley's Seedling (3n) ..			1								
Bramley's Seedling (3n) × Cox's Orange (2n)				1			1				
Blenheim Orange (3n) × Cox's Orange (2n)		2		1	3		2				
Blenheim Orange (3n) × McIntosh Red (2n) ..	1				3	2		1	1		
Totals	1	2	1	2	6	2	3	1	1		
Seedlings of Bramley's Seedling (probably 3n × 2n) .. (Data from Darlington and Moffett, 1930)		2	1	3	4		1			1	1
Total Number of Seedlings ..	1	4	2	5	10	2	4	1	1	1	1

number which is half the mean of the parental numbers, or else there is elimination of a proportion of those gametes and zygotes which have unbalanced numbers. Thus in the progeny of triploid × diploid apples one would expect to find the largest groups of seedlings with numbers of 42 and 43, since gametes with 25 and 26 in the triploid, and 17 in the diploid, should be most frequent. It will be seen, however, that the number 41 is the sum of the presumed primary haploid number, 7, and the secondary diploid number, 34.

It would seem most probable that the higher number of seedlings with forty-one chromosomes is due to the greater viability of gametes from the triploid with 17 + 7 chromosomes, thus combining the primary balance of seven with the secondary balance of seventeen.

TABLE III.

Chromosome Numbers of Seedlings from Crosses between Triploids.

	No. of Chromosomes															
	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
Blenheim Orange (3n) selfed				1												
Ribston Pippin (3n) selfed	1		1	1		4		3								1
Total ..	1		1	2		4		3								1

In the progeny of triploids selfed and triploids crossed by triploids a greater frequency of seedlings was found with chromosome numbers higher than the expected mean of fifty-one. Owing to the small numbers of these crosses it has been possible to examine so far, it is impossible to say whether the results are significant or not.

THE SIGNIFICANCE OF SECONDARY PAIRING.

Cytological evidence indicates that different degrees of affinity exist between the homologous chromosome sets of polyploids and that under certain conditions chromosomes which might be considered to be non-homologous are found to pair. (Collins and Mann, 1929 (1); Darlington, 1930 (5); Newton and Pellew, 1929 (10)).

It is probable that secondary pairing in the Pomoideæ indicates affinity between the chromosomes. The chromosomes have become sufficiently differentiated for a plant to behave, functionally at least, as a diploid with a basic number of seventeen, and the similar chromosomes within each of these sets have become sufficiently differentiated to preclude their regular pairing at prophase to form multivalent associations. Their affinity remains sufficient, however, to cause similar chromosomes to form groups and thus give rise to the phenomenon of "secondary pairing." The occasional occurrence of multivalents in diploids and tetraploids and their more frequent occurrence in triploids would be predicted on this basis.

Thus in diploid types the chromosomes almost invariably associate by prophase pairing to form bivalents, and later, by a secondary superimposed pairing of these bivalents, to form groups of four and six chromosomes each, these secondary associations being only seen at metaphase and anaphase.

The occurrence of autosyndesis within each of the sets of seventeen chromosomes in the Pomoideæ indicates that the primary basic number is less than seventeen, and the maximum association observed in "Northern Spy," where seven groups of chromosomes are found, seems to indicate a primary basic number of seven throughout the group. On such a hypothesis the haploid or gametic number of seventeen may be represented by the following formula as found for *Pyrus,* where A, B, etc. each represents a chromosome type:—

A A A
B B B
C C C
D D
E E
F F
G G

The chromosome constitution of the Pomoideæ cannot be interpreted on a primary basic number of eight (another common basic number in the Rosaceæ) since if such were the case never more than one group of six secondarily paired chromosomes would be found ; whereas two and three groups occur commonly. Further evidence is derived from the progeny of triploids (see Tables II. and III.).

In the Pomoideæ it is probable that the new chromosome constitution—secondarily balanced relative to the balanced constitution found in other groups of the Rosaceæ—has given rise to the characteristics distinctive of the group. Under experimental conditions an unbalanced form has always been found to differ from its progenitors in morphological characters. That such a form can be a success as regards viability and fertility is shown by *Dahlia Merckii* (Lawrence, 1929 (8)).

As a group the Pomoideæ are clearly distinguished from the rest of the Rosaceæ. Within the group the genera show, in certain respects, a high degree or morphological similarity, for example they have the same specialised type of fruit. It is plausible that such a specialised type of fruit may owe its origin to the change over to a new type of chromosome organisation.

SUMMARY.

1. The basic chromosome number throughout the Pomoideæ is seventeen. Diploid, triploid and tetraploid forms have been found.

2. At the first metaphase of pollen mother-cell divisions in diploids, secondary pairing of chromosomes is found throughout the group, indicating an affinity between chromosomes within each set of the diploid. Secondary pairing also occurs in triploids and tetraploids.

3. Multivalent associations are found in the diploids, and correspondingly higher associations in triploids and tetraploids, as would be predicted on the ground that secondary pairing indicates homology between chromosomes.

4. In seedlings of triploid × diploid apples the highest frequency is of forms with forty-one chromosomes. This is probably due to the greater viability of gametes with $17+7$ chromosomes in the triploid.

5. Chromosome morphology, pairing and breeding results indicate that, as in *Pyrus*, the thirty-four chromosomes of the diploid Pomoideæ are of seven types, of which four are represented four times and three are represented six times.

6. The number seventeen is therefore a secondary basic number and the derived series of polyploids (2n 34, 51, 68) are secondary polyploids.

7. The Pomoideæ may possibly owe their special morphological characters to the reconstitution of their chromosome complement, and it is suggested that such a change of balance is a factor in evolutionary change.

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CHEMICAL INVESTIGATIONS RELATING TO POTASSIUM DEFICIENCY OF FRUIT TREES.

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In previous investigations at this Station, it has been shown that potassium deficiency in fruit trees is an extremely serious problem in the important fruit areas of this country. As the result of this deficiency, fruit plants such as the apple, gooseberry, black currant and red currant exhibit the condition known as leaf scorch whilst certain varieties of plums, e.g., Purple Pershore, develop a chlorotic appearance in addition to showing the marginal scorching of the foliage.

In former publications, investigations relating to various aspects of the problem have been reported (2 to 7) and the data presented in the papers have included some chemical results illustrating the way in which potassium deficiency has been reflected in the composition of the plants in certain particular cases (3, 5, 6, 7).

Concurrently with the work already described relating to leaf scorch, comprehensive chemical studies were initiated, but the complete results have not been reported hitherto owing to the time required to complete the chemical determinations involved. The more important features of these results are summarised in the present paper.

In this branch of the investigations, it was considered desirable to determine the extent to which potassium deficiency is reflected in the various parts of fruit plants and to see how far observations relating to soil conditions, susceptibility of materials and remedial measures in cases of potassium deficiency could be linked up with chemical features of the plants.

In the course of this work, a very large number of cases of leaf scorch and of the type of chlorosis of plums previously shown to be due to potassium deficiency have been examined, and the chemical results obtained have demonstrated in every case that the scorched and chlorotic conditions of the plants are associated with chemical features typical of acute potassium deficiency.

Data relating to susceptibility of materials to leaf scorch indicate that resistant varieties and rootstocks may possess superior feeding powers for potassium than the more susceptible sorts.

The action of potash manures and of dung in rectifying the deficiency is clearly reflected in the chemical results and light is thrown on problems concerning the rate of observed responses to ameliorative treatments.

EXPERIMENTAL.

THE EFFECTS OF POTASSIUM DEFICIENCY ON THE VARIOUS PARTS OF FRUIT PLANTS.

At the commencement of the investigation, it was important to determine the extent to which potassium deficiency is reflected in the potassium content of various parts of fruit plants suffering from acute starvation of the element.

For this purpose samples consisting of leaves and stem portions of terminal shoots, spur leaves, bark and wood of young shoots and main stems, pulp of fruits and stones of fruits in the case of plums were examined from comparable healthy and potassium deficient plants.

Some typical results which illustrate the salient features revealed by the analyses are presented in Table I. (a), (b), (c) and (d).

The data reported in I. (a) are from an experiment in which apple trees, variety Stirling Castle on Malling Type I. rootstock, were grown in pots in sand, some of the trees receiving a complete nutrient solution and others a nutrient solution from which potassium was omitted (6).

The data presented refer to leaves and stem portions of terminal shoots and four-year-old main stems, and show the effects of the deficiency on the contents of dry matter, ash and potash in the various parts considered.

TABLE I.

Showing the Effect of Potassium Deficiency on the Potassium Content of the various parts of Fruit Trees.

(a)

Chemical Data.	Leaves of Terminal Shoots. %	Stems of Terminal Shoots (Winter Stage).*		Main Stems above graft union—4 years old.*	
		Bark. %	Wood. %	Bark. %	Wood. %
Complete Nutrient Series :					
Dry matter in Fresh Wt.	33.92	41.61	48.89	39.71	48.84
Ash in Dry Matter ..	8.91	6.19	2.28	6.19	1.13
K_2O in Ash	45.40	37.83	38.68	35.85	31.12
K_2O in Dry Matter ..	4.046	2.342	0.8818	2.219	0.3518
Potassium Omitted Series :					
Dry Matter in Fresh Wt.	38.00	46.19	53.96	46.67	53.56
Ash in Dry Matter ..	7.50	5.57	1.51	5.64	0.70
K_2O in Ash	10.58	16.37	18.61	16.77	20.68
K_2O in Dry Matter ..	0.7936	0.912	0.2809	0.9458	0.1448
K_2O in Dry Matter as % of K_2O in Complete Nutrient Series	**19.62**	**38.94**	**31.85**	**42.63**	**36.48**

* Data on material during third season of experimental treatments.

(*b*)

Manurial Treatment Annual Spring Dressing from 1912.	Farmyard Manure at 20 tons per acre.				Complete Artificials: Sulph. Amm. 1½ cwts., Super 5 cwts, Sulph. Potash 2½ cwts				Nitrate of Soda 1½ cwts.				Nitrate of Soda 1½ cwts. from 1912 + Sulph. Potash 2 cwts. from 1923.			
	Terminal Shoots.				Terminal Shoots.				Terminal Shoots.				Terminal Shoots.			
Chemical Data.*	Leaves. %	Bark. %	Wood. %	Fruit. Pulp. %	Leaves. %	Bark. %	Wood. %	Fruit Pulp. %	Leaves. %	Bark. %	Wood. %	Fruit Pulp. %	Leaves. %	Bark. %	Wood. %	Fruit Pulp. %
Ash in Dry Matter	6.81	8.53	2.51	2.10	6.86	9.53	2.64	1.00	5.87	8.91	2.68	0.90	6.66	—	—	1.16
K_2O in Ash ..	29.05	14.11	19.04	56.30	22.52	10.06	16.10	56.50	10.73	7.49	11.91	48.40	14.77	—	—	56.2
K_2O in Dry Matter	1.98	1.20	0.48	1.18	1.54	0.96	0.43	0.90	0.63	0.67	0.32	0.44	0.98	—	—	0.90
K_2O in Dry Matter as % of K_2O in Dry Matter of Complete Artificials Plot ..	**128.5**	**125.0**	**111.6**	**131.1**	**100**	**100**	**100**	**100**	**40.91**	**69.79**	**74.40**	**48.90**	**63.63**	—	—	**100**

* Data on Shoots, 1927, on Fruits, 1928.

(*c*)

Manurial Treatment Annual Spring Dressing from 1921.	Farmyard Manure at 10 tons per acre.			Complete Artificials: Nitrate of Soda at 50 lbs. N., Super at 120 lbs. Total Phos., Sulphate of Potash at 100 lbs. K_2O per acre.			Complete Artificials less Potash.		
	Terminal Shoots.			Terminal Shoots.			Terminal Shoots.		
Chemical Data.	Leaves. %	Stems and Petioles. %	Fruit Pulp. %	Leaves. %	Stems and Petioles. %	Fruit Pulp. %	Leaves. %	Stems and Petioles. %	Pulp of whole Fruits. %
Dry Matter in Fresh Weight	36.20	36.00	10.95	37.20	34.40	10.65	40.25	38.80	11.07
Ash in Dry Matter	8.64	5.65	3.83	7.78	5.25	3.64	8.40	4.73	2.94
K_2O in Ash ..	23.28	22.55	46.30	22.78	24.34	49.00	10.04	13.44	41.55
K_2O in Dry Matter	0.20	0.13	0.18	0.18	0.13	0.18	0.084	0.064	0.12
K_2O in Dry Matter as % of K_2O in Dry Matter of Complete Artificials Plot ..	**111.1**	**100**	**100**	**100**	**100**	**100**	**46.7**	**40.9**	**66.7**

(d)

Chemical Data.	Healthy Trees.			Potassium Deficient Trees.		
	Spur Leaves. %	Fruit Pulp. %	Stones. %	Spur Leaves. %	Fruit Pulp. %	Stones. %
Ash in Dry Matter	11.38	3.25	0.96	6.99	2.40	0.92
K_2O in Ash	18.51	53.15	32.10	3.78	44.90	32.69
K_2O in Dry Matter	2.11	1.73	0.31	0.26	1.08	0.30
K_2O in Dry Matter as % of K_2O in Dry Matter in Healthy Trees	100	100	100	12.32	62.43	96.77

The chief points to be noted, which are typical of the large number of cases examined, are that potassium deficiency is reflected in a very definite way in all parts. It results in relatively high dry matter in fresh weight, low ash content and low potash content in both ash and dry matter.

The value at the bottom of each column shows the extent to which the deficiency has lowered the potassium content in each case.

Table I. (b) gives data from a field manurial experiment on apple trees, variety Grenadier. The treatments, except in the fourth case, had been given for fifteen consecutive seasons before the samples of leaves and shoots were taken and for one more season before the fruits were examined. It should be noted that the trees on the nitrate of soda plot have been shown to be suffering from potassium deficiency by treating a portion of the plot with sulphate of potash as shown in the fourth case, the potash treatment having produced a marked growth response and resulted in the elimination of leaf scorch in three seasons.

The data also illustrate how farmyard manure has been effective in supplying the amount of potassium necessary to the trees, a result of which has also been obtained at other centres (See also Table I. (c)).

It will be noted from the results that potassium deficiency is clearly marked in the composition of leaves, bark and wood of terminal shoots, and in the pulp of the fruits.

The results reported in Table I. (c) are from a manurial experiment on gooseberries at this Station and for which data relating to the action of the various manurial treatments on growth, cropping, leaf scorch, etc., have been previously published (4, 5).

The data given in the present instance are provided for the purpose of showing how closely results with gooseberries resemble those in the case of apples.

Table I. (d) gives data in the case of a serious case of potassium deficiency of plum trees, variety Purple Pershore, and illustrates the manner in which the deficiency is reflected in spur leaves, fruit pulp and in the stones of the fruits.

It will be clear, again, that the deficiency has affected the ash and potash contents of the leaves and fruit pulp as in the previous cases, but it will be noted that the ash and potash contents of the stones have not been appreciably altered.

SOIL RELATIONSHIPS.

It has been previously emphasised that potassium deficiency usually occurs in the field in a patchy manner and in examining such cases it has been usual to find that soil differences can be recognised between healthy and " scorch " areas.

In addition to comparing the soil features in these cases, it has been usual to examine the composition of comparable portions of the healthy and " scorched " trees. Since potassium deficiency in the field usually leads to the cessation of terminal shoot growth, it has been customary to compare samples of spur leaves in cases of this character but in some cases samples of fruit pulp have also been analysed.

A very large number of cases have been investigated and, *in every one,* it has been found that the healthy trees were high potassium trees and the scorched trees of low potassium content, thus showing the patchy nature of potassium deficiency in the field.

Some representative cases from the bulk data have been selected to illustrate the point in Table II., and these clearly show the correlation between the soil patches and the potassium contents of the various plants.

SUSCEPTIBILITY OF MATERIALS.

Field observations relating to susceptibility of materials to potassium deficiency have been made in connection with classes of fruits, varieties and rootstocks.

Classes of fruits do not lend themselves readily to chemical studies of the character considered herein since opportunities of examining large numbers of varieties of different classes of fruits under comparable conditions do not normally occur and hence conclusions relating to susceptibility of different classes must be drawn from numerous less critical instances. For this reason chemical investigations relating to susceptibility have been confined to differences exhibited by varieties and rootstocks.

A case of differential varietal susceptibility in gooseberries is shown in Table III. where the more resistant variety Whinham's Industry is compared with the more susceptible variety Careless. In this particular case the Careless bushes had failed to make appreciable growth in three years after planting whereas the Whinham's, although exhibiting signs of potassium deficiency, measured on the average approximately 27″ to 30″ in height and spread.

TABLE II.

Showing the Relationships between Healthy and Scorch Areas and the Potassium Content of the Plants on them.

Soil Characters.	Class and Variety of Plant.	Healthy Area.			Scorched Area.		
		K_2O in Dry Matter.			K_2O in Dry Matter.		
		Leaves. %	Stems. %	Fruit Pulp. %	Leaves. %	Stems. %	Fruit Pulp. %
Keuper Marl Formation—fine sandy loam.	Apple—var. Lane's Prince Albert	1.94	—	—	0.42	—	—
Glacial Sand—coarse sand ..	Apple—var. Worcester Pearmain	0.69	—	0.66	0.37	—	0.42
Old Red Sandstone Formation—clay loam	Plum—var. Purple Pershore	2.26	—	1.39	1.33	—	1.17
Silurian—Old Red Sandstone mixture—close textured silty loam	Do.	2.41	—	1.41	0.40	—	0.55
Lias Clay with slight coarse sand Drift admixture—heavy clay ..	Do.	2.11	—	1.75	0.26	—	1.08
River Gravel—light sandy loam ..	Do.	1.77	—	—	0.41	—	—
Glacial Sand and Gravel—coarse sand and gravel mixture ..	Gooseberry—var. Careless ..	0.57*	0.61*	—	0.34	0.43	—
Lias Clay Formation—heavy clay	Gooseberry—var. Careless ..	2.90	—	—	1.66†	—	—

* This case bushes not healthy but less affected than on " scorched area."

† Severity only very slight.

It will be noted that the Whinham's contain higher percentages of potassium in both the leaves and stem portions than do the Careless bushes, though the percentages of K_2O in the ash of both portions of the Whinham's suggest that the bushes are growing under conditions of too low potash (c.v. values, Table I. (c)).

TABLE III.

Showing Varietal Effects in Relation to Potassium Deficiency of Gooseberries.

Variety.	Terminal Shoots.					
	Leaves.			Stems.		
	Ash in Dry Matter. %	K_2O in Ash. %	K_2O in Dry Matter. %	Ash in Dry Matter. %	K_2O in Ash. %	K_2O in Dry Matter. %
Whinham's Industry	10.37	6.17	0.64	4.73	13.82	0.65
Careless	8.90	3.86	0.34	5.20	8.39	0.43

TABLE IV.

Showing Rootstock Effects in Relation to Potassium Deficiency of Apple Trees.

Variety and Rootstock Type.	Material Analysed.	Ash in Dry Matter. %	K_2O in Ash. %	K_2O in Dry Matter. %
Worcester on Malling Type I. ..	Green Spur Leaves	9.41	5.29	0.50
Worcester on Malling Type V. ..	Scorched Spur Leaves ..	7.25	3.82	0.28

In Table IV. the data refer to a case in which the differential effects of two rootstocks are clearly visible, the trees on Type V. being obviously more seriously affected than those on Type I. At the time of collecting the samples, the foliage on the Type V. trees was mostly severely scorched whilst scorching was only present to a slight extent on the leaves on the trees on Type I. In taking the samples, leaves of healthy appearance only were collected from the Type I. trees and scorched leaves only from those on Type V. This method of sampling would possibly accentuate any differences in composition in the two cases though the samples were fairly representative of the two sets of foliage.

The values in the table show a definitely higher potassium content in the leaves from the Type I. trees, but here again the percentages of K_2O in ash and dry matter suggest that the trees are suffering from severe potassium starvation.

REMEDIAL MEASURES.

Examples of the effects of applying remedial measures in cases of potassium deficiency by means of potash manures and dung are illustrated in Table I. (b) and (c).

The data presented in Table V. are from an experiment carried out in a commercial plantation (7) in which a very marked response on gooseberries in a severe case of leaf scorch was observed as the result of two annual dressings of sulphate of potash at 4 cwts. per acre, whereas on red currants, also in a badly scorched condition, these dressings did not produce a visible reaction.

The chemical data show that the potassium content had been increased to a much greater extent in the case of the gooseberries than in that of the red currants. It should be noted that although no appreciable effect had resulted from the potash dressings on the currant bushes, the potassium content had been significantly increased.

TABLE V.

Showing the Difference in Rates of Response to Potash Manuring of Red Currants and Gooseberries.

	Red Currants.				Gooseberries.			
	Potash treated Plot.		Control Plot.		Potash treated Plot.		Control Plot.	
	Leaves. %	Stems. %	Leaves. %	Stems. %	Leaves. %	Stems. %	Leaves. %	Stems. %
Ash in Dry Matter ..	15.58	7.17	13.61	6.71	10.11	5.00	10.72	4.86
K_2O in Ash	13.08	10.95	6.42	7.82	23.03	27.24	4.45	6.69
K_2O in Dry Matter ..	2.04	0.79	0.87	0.52	2.33	1.36	0.48	0.32
K_2O in Dry Matter in Potash treated Material as % of Potash in Control Material	243.5	153.8	100	100	485.4	425.0	100	100

An interesting point is illustrated by the data in Table VI., which were obtained from an experiment on plum trees in which the trees on the potash deficient area had shown no appreciable response following three annual dressings of sulphate of potash at a rate of 3 cwts. per acre.

In the fourth season certain of the trees on the plot were sprayed with a 1 per cent. solution of sulphate of potash at intervals of approximately ten days throughout the growing season in addition to receiving a further spring dressing of sulphate of potash as in previous years.

TABLE VI.

Showing the Effects of 1 per cent. K_2SO_4 Spray on Plum Trees, variety, Purple Pershore, over one season, on composition of Foliage and Fruit.

	Healthy Green Trees.		Potash Deficient Trees—Sprayed + K_2SO_4 manures.		Potash Deficient Trees—Unsprayed + K_2SO_4 manures.	
	Leaves. %	Fruit Pulp. %	Leaves. %	Fruit Pulp. %	Leaves. %	Fruit Pulp. %
Ash in Dry Matter ..	9.97	2.60	6.35	—	4.83	2.20
K_2O in Ash	22.69	47.90	20.94	48.10	11.37	33.60
K_2O in Dry Matter ..	2.26	1.25	1.33	—	0.55	0.74

The spraying treatment produced obvious improvement in foliage and fruits whilst the further manurial dressing did not result in obvious response.

The analyses of the leaves and fruit pulp presented in the table show that the spraying treatment was effective in raising the potash content of the portions examined to a level well above that of the trees receiving only the manurial treatment. It is also of interest to note that the data for the "manured only" trees suggest that the manurial treatment has been ineffective as a means of remedying the condition of potassium deficiency.

DISCUSSION.

The results reported in the various sections of this paper show the value of making chemical analyses of various portions of fruit plants in investigating problems of potassium deficiency.

Deficiency of the element is reflected in the leaves, in bark and wood portions of branches and stems and in the pulp of fruits, and by studying the potassium status of these, the condition of the plants can be gauged and the effects of ameliorative treatments followed.

The data relating to soil relationships show clearly the patchy distribution of soil areas conducive to potassium deficiency and illustrate the value of such symptoms as leaf scorch for diagnostic purposes.

It is clear, from the data in Tables II. and IV., relating to susceptibility of varieties and rootstocks, that though the differences exhibited are significant they are not of an order sufficiently large to solve the problems of potassium deficiency solely by the choice of resistant varieties and rootstocks. Even in notable cases of resistance, the potassium content has been much below the point produced by adequate manurial treatment.

Chemical examination of the plant is obviously of great value in experiments in which the results are not easy of interpretation, as in the cases of lack of response to manurial treatment such as are illustrated in Tables V. and VI.

In the former case the chemical results indicate that the two types of plants considered respond at different rates to the treatments given, and in the latter it is apparent that the ordinary method of applying potash manures is not an effective means of obtaining a response in a reasonable period under the prevailing conditions.

Similar cases of lack of response to potash manuring in cases of potassium deficiency of prune trees have been recorded in America (1). It seems probable from the evidence that the lack of response in such cases is due to the applied potash being unable to penetrate to the root systems owing to the absorptive properties of the soils concerned.

SUMMARY.

1. An account is given of chemical investigations which have been carried out in connection with problems relating to potassium deficiency of fruit trees.

2. It is shown that deficiency of potassium produces definite effects on the composition of various portions of the plants.

The deficiency is reflected in the leaves, shoots, main stem and pulp of fruits, but not in the stones of plums.

Percentage dry matter in fresh weight is usually increased, and ash content and potash content in ash and in dry matter are greatly decreased.

3. Soil areas conducive to potassium deficiency occur in patchy fashion in the field. They can be distinguished either by symptoms such as leaf scorch or chlorosis, or by chemical examination of the trees.

4. Observed differences in susceptibility to leaf scorch of varieties and rootstocks are accompanied by differences in potassium content of the plants concerned, but the chemical differences are too small to suggest that potassium deficiency in the field can be overcome entirely by the use of resistant materials of the nature examined.

5. The action of dung and potash manures in rectifying potassium deficiency is reflected in the composition of the treated plants. The analysis of appropriate portions of plants provides valuable information in attempting to interpret the responses following the application of remedial treatments.

In cases where negligible responses were obtained from potash manures, it is shown that the plants had failed to take in adequate amounts of potassium from the dressings applied.

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OLD AND NEW STANDPOINTS ON SENILE DEGENERATION.

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Every fruitgrower is acquainted with the so-called " Degeneration " problem. To some a friend will have pointed out his cankered apple trees as being beyond all saving owing to " degeneration " ; others may have been present at lectures, where continued vegetative propagation was blamed for the deterioration of fruit trees ; or again another may have had his attention drawn to it by a claim in his gardening paper for a new pear or apple that " it is only recently raised from seed and so does not show such defects of age as canker, scab, etc." Or again the horticulturist may note rows of fine, healthy trees, laden with fruit, of varieties thought to have degenerated in other parts of his country. Or, if he goes abroad on business or pleasure, he will find there whole orchards of fruit varieties, which have entirely disappeared in his own land, apparently owing to old age.

As a result few persons have settled convictions on the matter. To resort to books or to growers or botanists for an answer is only to be confronted with endless controversy, the arguments being seemingly pretty equally divided for or against, backed moreover by learned comparisons between plant and animal life, the value of which it is very hard to gauge.

A review of the most important literature on the subject can be found in a recent number (No. 12) of the Mededeelingen van het Laboratorium voor Tuinbouwplantenteelt, Wageningen, Holland, where the author of this article discusses the matter in connection with investigations carried on at the Laboratorium voor Tuinbouwplantenteelt (Department of Horticultural Research) of the Wageningen Agricultural College. As this lengthy publication appeared in Dutch, with only a summary in English, which Mr. R. G. Hatton had the kindness to look over before it appeared, it is not readily available to English-speaking horticulturists. Mr. Hatton suggested, therefore, that an abbreviated English version should appear in the Journal of Pomology.

The degeneration theory is founded on the supposed analogy between the individual in its strictest sense (a man, a cat) and the total living material which originated with one fertilization and which was propagated consecutively by vegetative means. This mass of material, or collection of minor individuals, is called a clone. Thus all the representatives of any one variety of the Damson Plum, all the plants of one potato variety together form a clone.

Since, generally speaking, living organisms weaken with old age, many naturalists expect the same senility to appear in clones too. The longer a clone has existed, the more it is supposed to suffer from various ailments.

If this supposition is true, two cardinal points require elucidation:

(1) Is the duration of life in the individual dependent on external or internal causes ?

(2) Will a clone continue to live indefinitely or will it die out ?

In the above publication these questions have been considered, first from the standpoint of the zoologists and biologists, as far as their results contribute towards a solution of the horticultural problem.

The answer to the first question is nearly unanimous. An animal dies in consequence of internal conditions, inherent in life. Many theories have been published about the nature of these conditions, some of which are briefly discussed.

Hertwig supposed that the nucleus would become smaller during the life of the cell. The relation of the nucleus volume to the cell protoplasm would be an index of the vitality, and this index would become progressively worse in old age, until death resulted.

Bühler saw in the degeneration of cells the consequence of chemical action in the living protoplasm.

Child (1915) lays stress upon the process of differentiation, which involves a decrease in the proportion of chemically active protoplasm.

Another popular theory is that wearing of the vital organs of the body causes senility. Among these organs the heart and lungs would be foremost, as their highly specialized cells lack the power of regeneration. This " wearing-out " theory is supported by the fact that in some species of lower animals life can be suspended for long periods, often for years, and afterwards resume its normal course. In such cases the death of the individual has been postponed for a considerable length of time. Schleip gave as an example some muscle trichinæ, which were thus kept alive for thirty years.

The " wearing-out " theory offers, however, no explanation for the cessation of cell-regeneration, which is the final cause of death.

The famous scientist Metchnikov advances the theory that certain products of metabolism, formed in the large intestine, slowly poison the living body.

The diminishing speed of growth, very clearly visible in the weight-curve of living beings, attracted the attention of Minot. He found that with increased specialization and differentiation growth decreases, and that highly specialized cells even lose their capacity for division. As only specialization creates the possibility for highly developed mental and physiological processes, death seems to be the price we have to pay for life, at least for life as we know it in man and in the higher animals. Unicellular organisms, being hardly differentiated, alone escape payment.

The problem of whether a clone can degenerate has been attacked by many investigators of such organisms ; only some of the most important publications on the subject can be mentioned in this article.

Paramœcium, a protozoon commonly found in stagnant water, has been a most fruitful subject for research, as it is capable of both sexual and asexual reproduction, and will produce many generations in a short time. When conditions of nutrition are kept on a high level, sexual reproduction can be avoided by repeated transplantation of an individual to new nutrient solution, and a clone originates by continual cell division.

Maupas, who studied the conjugation of about twenty different *Infusoria*, observed that after a longer or shorter lapse of time all clones die out, in consequence of decreased reproduction and decreased absorption of food from the nutrient solution. A single conjugation seemed to correct these failings.

Enriquez (1903) was the first investigator to suggest that so-called "degeneration" is merely a result of unfavourable circumstances. He succeeded in raising 863 generations of *Glaucoma* without a single indication of senility.

During the last ten years improved methods of culture have to a large degree borne out Enriquez' supposition.

Hartmann had similar results with a Flagellate, *Eudorina*, 3,500 healthy generations being grown during a period of twelve years.

Better still were Metalnikov's results. His cultures date from the year 1908, and up to 1924 he had raised approximately 5,000 asexual generations of *Paramœcium caudatum*. His figures do not show any decrease in the rate of reproduction.

The largest numbers were attained by Woodruff, who started his experiments with *Paramœcium Aurelia* in 1907, the following table showing the number of generations per year and per day:

In 1907	452	generations,	day average	1.24	generations
1908	690	,,	,,	1.89	,,
1909	613	,,	,,	1.68	,,
1910	612	,,	,,	1.67	,,
1911	662	,,	,,	1.81	,,
1912	692	,,	,,	1.89	,,
1913	671	,,	,,	1.84	,,
1914	679	,,	,,	1.84	,,

Total 5,071 generations: Total day average 1.74 generations.
Total year average 634 generations.

At the close of the year 1920 Woodruff had reached the 8,400th vegetative generation, and in his latest report (1926) he mentions that the 11,700th generation is as vigorous as the first one in 1907!

Clearly the *Infusoria* are not the only group of animals where sexual reproduction may be dispensed with ; in the bacteria a sexual process is unknown and yet no sign of degeneration is seen.

VEGETATIVE CLONES: ARGUMENTS FOR AND AGAINST THE THEORY OF "SENILITY."

The advocates of senility in higher plants mainly believe in a strong analogy between the clone and the individual in its strictest sense. According to them the graft or cutting remains part of the parent plant which forms, together with all its vegetative descendants, one single individual of equal age. This whole clone grows old contemporaneously with the parent plant. Since only a sexual process rejuvenates, all the minor individuals of a clone should consequently die of old age at the same time, though this perfectly logical conclusion has not been drawn by many authorities. Usually it is qualified by allowing for a slight rejuvenation due to external influences ; only five authorities (Witt, Verschaffelt, Fliess, Vischer and Prinsen Geerligs) mention this extreme consequence.

Vischer sets out the problem more clearly by posing certain definite questions :

> What age can a clone attain ?
>
> Is the vegetative offspring constantly rejuvenated on separation from the parent plant, and does it thus become independent of its age ?
>
> Or does it remain part of the organism from which it is separated in space, partaking of its age, and ending life at the same time, no matter when its separate existence started ?

Molisch and some other authorities distinguish between natural and unnatural vegetative reproduction. The first method, including multiplication of bulbs and potatoes, cutting and layering of plants in which root formation is very easy, would, they hold, cause rejuvenation in the offspring. The second group would then include such horticultural practices as grafting, oculating (budding), etc., where the new plants would presumably remain as old as the parent plant.

This standpoint does not seem justified, because the logical basis of senility, the lack of a sexual process, loses its significance, and because no line can be drawn between natural and unnatural propagation.

Many other botanists are opposed to the theory of senile degeneration. They consider cuttings or grafts as physiologically independent units, possessing in common with their parent plant the hereditary factors alone. A clone, moreover, is a physiologically heterogeneous complex of individuals, and there is no reason why physiological death should occur at the same time in all. They hold, then, that the clone is persistent, provided that sufficient new representatives are propagated.

HISTORY OF THE "DEGENERATION" THEORY.

The first authority to mention "senility" in plants was Parmentier in the year 1786, while discussing the vegetative propagation of potatoes. Anderson (1795) opposed the idea, and explained decreased production by a lack of selection. Knight, the well-known fruitgrower, was the third author to mention senility, and the first to apply the hypothesis to various groups of cultivated plants.

Van Mons (1835, '36) popularized the idea, and in his successful work of raising new fruit varieties followed a method based on the senility theory.

As a result of Knight's and Van Mons' activities the majority of horticulturists became adherents of the theory, since it seemed to explain the debilities and diseases of their crops. During the latter half of the nineteenth century a heated controversy arose in periodicals and publications, but very few definite conclusions were reached.

"Degenerated" varieties were found among apples, pears, prunes, potatoes and strawberries, as also among roses, red currants, poplars, weeping willows, grape vines, Hevea, sugar cane, Canadian Waterweed (*Elodea*), even among perennials. Recently the common reed (*Phragmites*) and the elms in Holland have been added to the list. At present the controversy has subsided, though occasional references to it occur in horticultural periodicals. No agreement has ever been reached, though many cases of supposed senility have actually been traced to plant pests.

Although Benedict set the question on an experimental basis in the twentieth century, its complexity has prevented any conclusive answer up to the present. We may well agree with a writer in *The Gardener's Chronicle* of 1875: "Nor do we think it at all likely that our knowledge is sufficient to enable us to give a definite answer to it at the present time."

SENILITY IN POTATOES.

As mentioned above, potatoes were the first plants to be suspected of senile degeneration. Reduced yield per acre and strong attacks of *Phytophthora* and other diseases were considered as symptoms, especially in the varieties (clones) Dabersche, Munstersche, Echte Lange Kartoffel, Imperator, (Sutton's) Magnum Bonum, Weisse Zwiebel and Champion. In Germany, especially, the degeneration theory found many strong supporters, who considered fifty to sixty years the maximum age of a potato clone (Thuer). Others, e.g., Morstatt, even mention thirty years as the span of life.

Now the Magnum Bonum clone was imported into Germany in 1878; in 1891 it is quoted by Heine as degenerated. In Holland it was not successful, owing to severe attacks of *Phytophthora* and a virus disease, but at the beginning of the twentieth century its yield in some parts of Germany was still

31,000-42,600 Kg. per Ha. (approximately the same in pounds per acre). In 1910 Magnum Bonum was the second most important potato in the Belgian district of Condroz (with 22,800 Kg. per Ha., second only to Athènes with 23,470 Kg. per Ha.). Salaman, who quotes the disappearance of Magnum Bonum in England (1921), refuses to agree that senility is the cause, seeing that the variety is still vigorous in Sweden, as also nowadays in Denmark. Thus, forty years after Heine had pronounced it degenerated, it still remains one of the chief crops in various countries.

The potato Suriname, which, as early as 1795, was praised as specially resistant, was still in cultivation in the year 1921. Here the span of life must be at least 126 years!

The first attempt towards the establishment of a mathematical basis for the discussion of senility in the potato were made by Ehrenberg and Tuckerman, who used yield-per-acre figures, given by the German Potato Experimental Station and by the grower Heine. Both authors concluded, apparently independently, that no indication of degeneration could be found in the varieties at their disposal. Although discontent with old varieties is common, this is mostly due to changing economic conditions.

Nowadays all these " symptoms of senility " are known to be caused by infectious diseases, among which virus diseases are prominent ; Quanjer and Oortwyn Botjes have been pioneers in this field. Salaman ventures : " In a word, immunity to mosaic is the key to immortality in the potato."

In Germany, however, some well-known authorities still cling to the senility idea. Among them Graebner (in Sorauer-Graebner, 1921 and 1924) must be mentioned, in his revision of Sorauer's Handbook of Plant Diseases, though Sorauer himself declared as early as 1909 that it was not the plant but the soil which was degenerated.

SENILITY OF FRUIT VARIETIES.

As the readers of the JOURNAL OF POMOLOGY will probably be more interested in this part of the problem, we will discuss the matter in regard to fruit trees somewhat more extensively.

Any number of fruit varieties have been accused of senile degeneration, and in various publications we find forty-one pears, thirty-six apples and a plum listed. Among these are the well-known varieties Louise Bonne d'Avranches, Golden Pippin, Gravenstein and Reinette de Canada. If we ask the supporters of the degeneracy theory as to the precise symptoms shown by senile fruit trees, we get the following answer :

On the fissured bark of trunk and branches canker is to be found ; the leaves wilt and die, and offer a shelter for insects. In the crevices of the bark, the eggs and larvæ of insects are abundant, while the adult insects feed on the sap which flows out of the open wound. Mosses cover the trunk. Other symptoms exhibited are tardy and irregular fruiting or complete sterility, shedding of flowers, abundance of apple maggots, and gummosis.

Similar symptoms are cited by Van Mons, Wttewaal, Fischer, Puvis, Zorn, Pynaert and other nineteenth century authorities.

From the mass of literature on the subject we can only quote from one or two of the most important articles.

Thomas Andrew Knight remarked in the neighbourhood of Elton in Herefordshire many orchards with badly cankered fruit trees sometimes covered with mosses. He tried to improve conditions by grafting twigs from the best trees on young stock, and subsequently by grafting or oculating (budding) several times in succession, being bent on severing every connection between the graft and the old parent tree. To his astonishment the greater part of the vegetative descendants proved just as susceptible to diseases and pests as the original trees ; three years afterwards all the young trees were covered with mosses. For Knight this was an indication that the grafts were just as old and degenerate as the parent trees and had very soft wood, which made them susceptible to diseases. This change in the quality of the wood is "probably . . . the effect of old age and therefore incurable."

We know now that hard or soft wood is a factor of very little importance, and that most of the ailments of fruit trees which Knight describes are due to climate and to lack of care and precaution on the part of the fruitgrower. It must also be remembered that the use of fertilizers and sprays was still unheard of.

Knight's work may therefore be considered as an interesting historic document, important to us only because he was the first to mention senility in relation to fruit varieties.

Jean Baptiste van Mons popularized Knight's ideas both by raising and setting "young and vigorous" varieties and by his book "Arbres fruitiers. Leur culture en Belgique et leur propagation par la graine, ou Pomonomie belge." (Fruit trees. Their cultivation in Belgium and their propagation by seed, or Belgian Pomonomy.) The first part appeared in 1835, the second in 1836. The enormous influence of van Mons is shown by the fact than in 1880 a Dutch nurseryman mentions in his catalogue no less than 35 original varieties raised by van Mons, some of which are still popular, e.g., Beurré d' Amanlis, Beurré de Mérode (Double Philippe), Conseiller à la Cour, Nouveau Poiteau and Calabasse Bosc.

Van Mons explains the "senility" of old varieties by the exhaustion of the vital energy provided by the original seed. The propagation of a "senile" clone by seed will immediately bring back the old vitality. In his methods of raising varieties van Mons did not apply his own theory, for he sowed repeatedly before he was sure of having "rejuvenated" the stock sufficiently. Many other contradictions are to be found in his book, one of the most remarkable examples being his accounts of the cause of senility. In the first part of the book he cites abundant blossoming and heavy bearing as causes, while in the second part only the age of the clone is mentioned.

Puvis, another supporter of the degeneration theory, is convinced of the great importance of fecundation, the only process to cause rejuvenation. Nevertheless, he believes that a slight rejuvenation occurs every time a variety is grafted on young seedling stock. This deviation from the fundamental thesis of senility serves to explain the fact that healthy trees of a degenerated variety may be found many years after the death of the original tree.

This reservation of Puvis may have been necessary, for some varieties are exceedingly old ; Zorn relates that the Edelborsdorfer was already known in the year 1247, and according to Jablanszy (1911) Ribston Pippin was a popular variety as long ago as 1709.

Hedrick, the well-known American pomologist, wrote in 1928:

> " . . . in writing up the histories of several thousands of fruits for the fruit-books of New York, I have never come across any evidence whatsoever that varieties have changed in the least for better or worse, and a few of our kinds as the Damson Plum and the Pound Pear and possibly several others go back quite to Christ's time, and were described by Pliny at that time. They seem to be now exactly what they were then."

Others lay emphasis upon the lack of degeneration in other asexually propagated plants (e.g., banana, yam, date, olive, fig, sweet potato and *Acorus Calamus*), on the original presence of defects in some varieties like White Winter Calville, which were considered as examples of degeneration because of these defects, and lastly upon the success achieved with " antiquated " varieties in regions possessing different, often better soil and climate. The Gravenstein apple, which is still one of the four most important commercial varieties in the north-western parts of the United States, affords a good instance.

The use of the word " degenerated " has led to confusion. It has been used alternatively in evaluating the variety and in explaining the cause of its condition. It ought not to be used in an explanatory sense. The explanation of " degeneration " of fruit trees has been found to lie in frost damage, in neglect of orchards (Speechley, *Gardener's Chronicle*, 1875: " Apples properly planted will retain their good qualities so long as sun and earth endure "), in adaptation of insects to new foster plants (Quanjer), in lack of selection or in faulty selection (Sprenger), and in modification of plants.

A very important cause of apparent "degeneration" is the higher standard by which our fruit is judged nowadays. We expect of first grade fruit, besides good size, appearance, weight and flavour, several qualities valuable in shipping, such as a smooth, strong skin and flesh which does not bruise easily. Half a century ago apples and pears were consumed in the immediate vicinity of the orchard, and provided the flavour was good, size and appearance did not matter so much. Nowadays, small, delicate kinds like the Courtpendu's and the Bergamottes have hardly any market value.

As a result of Van Mons' work and the acceptance of the senility theory a group of nurserymen and amateurs in the Gelderland province of the Netherlands started a campaign for growing apples and pears from seed. Their aim was to obtain vigorous young clones, approximating rather to the old types than entirely new varieties. This aim was not reached, of course, but some types then originated have become popular in Holland, and are widely planted, e.g., the Notarisappel, the Lemœnappel, the Luntersche Pippeling and the Beurré van den Ham. Though this movement of the Luntersche Tuinbouwvereeniging (Horticultural Society of Lunteren) has not spread so widely as the founders wished, it stands out in the wilderness of publications as a case where a group of people did something, instead of only discussing the matter. The work does not offer a good example of methods in breeding, no precautions against insect pollination being taken, and it is only because the society had the choice of many thousands of seedlings that plants were found which were less " degenerated " than the parent-trees.

THE LOMBARDY POPLAR.

The Italian Poplar, a native of the Crimea and Asia (Near East, Persia, Himalayas), was imported into Poland at the close of the seventeenth century and introduced into Italy in the middle of the eighteenth.

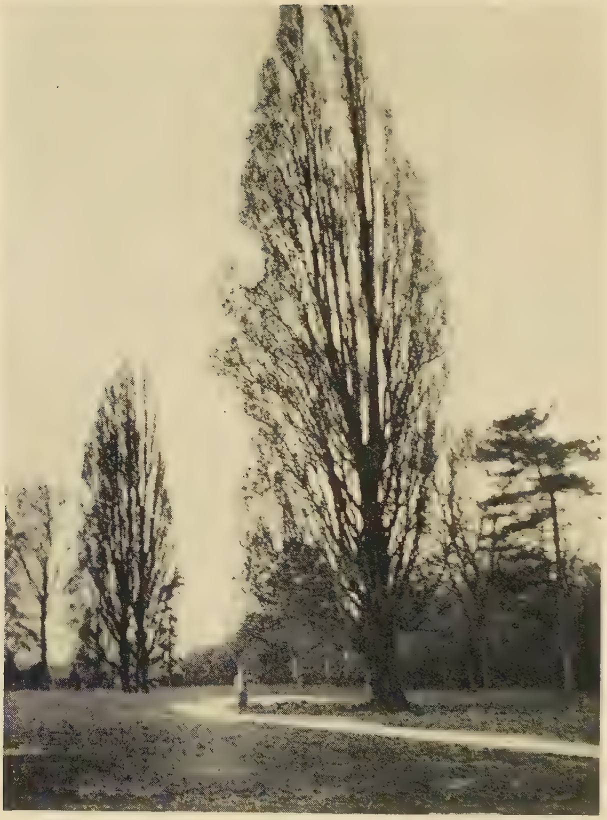
From Lombardy it found its way to Germany, where the first trees were planted at Wörlitz and Dessau. In France its introduction was probably due to Napoleon, who had many highways planted with it. The first tree in England was brought over in 1758, by the Earl of Rocheford.

It is a remarkable fact that nearly all Lombardy poplars in Europe are male, and consequently have always been propagated by cuttings. Only a few female trees have been found ; Hampl mentions in 1893 trees at Frankfort on Oder, Braunschweig, Schwetzingen, Karlsruhe and Neudorf in Bohemia. Hegi adds to this list Freiburg i.B., Waldenheim near Strassburg and Vienna.

The reason for this preponderance of male trees may be found in the fact that their growth is more slender than that of the females.

In the nineteenth century many reports were current of Lombardy poplars having dead tops and dying branches ; between 1820 and 1840 in England, in 1840 in America, and in the Netherlands and the northern and central part of Germany during the latter part of the century.

Senility was considered the cause of the phenomenon and was at the time



Old Lombardy Poplars in the Bois de Boulogne, Paris.

a perfectly logical explanation, fitting in beautifully with the continuous asexual propagation of the poplar.

According to many authorities the weakened clone may be expected to offer insufficient resistance to frost and to some fungi, *Dothiora* and *Didymosphæria.*

Others regard these fungi as the primary cause, and point to the fact that attacks occur especially on dry soil, and that trees are perfectly sound in regions with a milder climate, such as the valley of the Rhine, Bohemia, Italy and Southern Germany.

In Flora and Sylva, 1904, we read, " . . . Many trees are diseased, especially where the situation is not well chosen for them. . . . In the north of England and in Scotland the Lombardy poplar does not thrive, but in valleys of the midland counties fine trees may be seen, particularly in Oxfordshire, where some of the oldest trees in England are still standing."

Neither did degeneration occur everywhere in France. The photograph, taken in April, 1930, shows several beautiful, healthy Lombardy Poplars in the Bois de Boulogne, Paris.

Apparently it is unnecessary to attribute the local shortcomings of the Lombardy poplar to senility of the clone.

WEEPING WILLOW.

A certain amount has been published on senility in the Weeping Willow. No discussion can be ventured, however, because it is quite uncertain which willows the writers refer to in speaking of the Babylonian Willow. *Salix babylonica* proper is not hardy in Western Europe and is very rarely found. Nurseries stock under that name a number of hybrids and weeping forms of other species.

GRAPE VINE.

When the senility idea was at its height, the grape was also supposed to show degeneration, especially in its susceptibility to *Oidium Tuckeri, Peronospora viticola* and *Phylloxera vastatrix.* But since the introduction of spraying to check mildew and the use of American stocks for European grapes to prevent the attacks of *Phylloxera,* no further accounts of senility have come to hand, though the same varieties are in use as formerly.

ELMS.

Clone degeneration was suggested by Smits van Burgst (1925) as the cause of the threatened disappearance of elms in Germany and Holland, which was actually due to a disease outwardly similar to the Chestnut Blight, and he urged the growing of young, vigorous plants from seed. He was, however, alone in this opinion, and since then the cause has been definitely proved to be *Graphium ulmi.*

ROSES.

The "La France" rose, brought out by Guillot in 1867, proved to be unsatisfactory for forcing at the beginning of this century, both in Germany and Holland. Witt, Fliess and Graebner attributed its susceptibility to fungi and its meagre flowering to a general degeneration of the clone, an idea which was attacked by Von Schwerin.

Many rose growers still grow this variety and have perfectly healthy plants, and in the Southern States of North America and in Cuba it is still an extremely popular garden-rose. These facts indicate that the history of La France will probably not serve as an argument in favour of "senility."

A number of other garden plants have been cited as degenerated, such as the Lübecker Currant, the Vierländer Strawberry (Brick) ; Thuer even mentions that many perennials, among which are *Chelone barbata, Helenium Hoopesii, Erigeron aurantiacus, Erinus alpinus, Leontopodium alpinum* and *Achillea moschata* run out after five or six years by reason of senility. Again this opinion is unsupported by other authorities and uncorroborated by observations in gardens and nurseries.

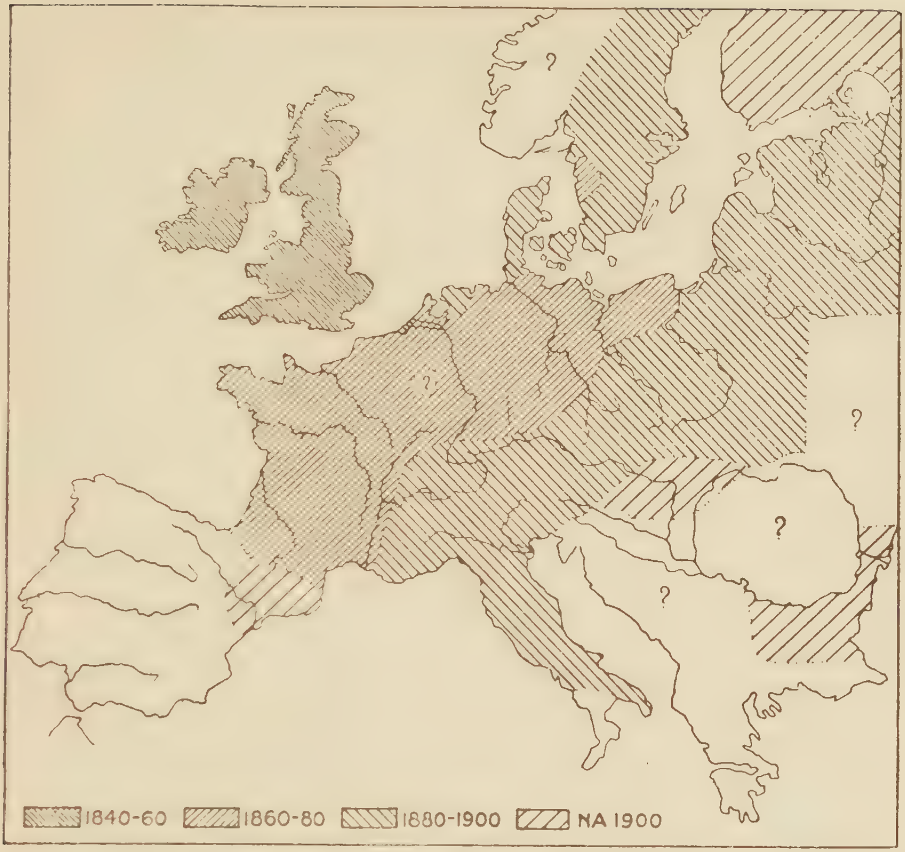
ELODEA CANADENSIS.

The Canadian Waterweed has been naturalized in Europe since the middle of the nineteenth century, but only female plants have been discovered. For nearly a century the clone has grown asexually, and for that reason it has been cited by opponents of "senility," like Möbius and Kirchner, Loew and Schröter. Supporters of the "senility" theory, on the other hand, cite the history of this plant as supporting their views (Sorauer-Graebner, Von Fliess and Agnes Arber) on the ground that, whereas at first its growth was so vigorous and increase so rapid that brooks were blocked and navigation impaired, yet some years afterwards the plant, though usually present in the same localities, was not troublesome.

The map shows the spread of the plant over Europe, in twenty year periods according to the literature on the subject.

Very little is heard nowadays of *Elodea* and unfortunately we do not know whether it is still growing and flourishing at the fringe of its habitat, as it was eighty years ago in Western Europe.

Undoubtedly, for reasons unknown, *Elodea* has diminished in the older parts of its habitat. Some authorities suggest an exhaustion of the necessary food supply (Staring, Bruinsma), others suppression by *Azolla*. Before a decision in favour of "senility" being the cause can be made, a new study of the *Elodea* problem seems necessary. The history of *Azolla* is in many points similar to that of *Elodea*, for there too we find a rank growth at first, subsiding



Distribution of *Elodea canadensis* in Europe.

later. *Azolla*, however, has a sexual process which precludes the possibility of degeneration due to asexual reproduction.

HEVEA.

Hevea has largely been propagated asexually since the beginning of this century, and according to recent issues of technical periodicals we find mention of the senility idea. Maas (1919) doubts if senile degeneration exists ; Vischer (1921) does not express a positive opinion but leaves the possibility an open question. Donkersloot (1921) is sure senility exists, and expects increased susceptibility to diseases, quite incorrectly mentioning the purple beech as an example.

SENILITY IN SUGAR CANE ?

Since 1850, when Pritchard gave it as his opinion that the sugar cane had degenerated by reason of old age, the hypothesis has been under constant discussion. It was dealt with at the Sugar Congress in Java, 1905. Discussions during and before the tenth Sugar Congress in Sourabaia, 1928, were even more interesting. One member only was convinced of the existence of senile deterioration in the sugar cane. Certain others who leaned toward the same point of view, but did not accept all its logical consequences, gave figures of sugar cane clones which seemed to show the influence of senility ; in every case, however, their data were corrected by others, and no proof was given.

The chairman concluded that the yield of a sugar cane variety depends on external factors, which may cause either an increase or a decrease. It was impossible to determine positively whether an irreversible decrease in vitality (degeneration of the clones) actually occurs.

That the main varieties do not show any decrease in yield is shown by the following table, which gives the production of some well-known Java canes for the period 1912-28 (in pikols standard muscovado per bouw, a pikol being 138 lbs. and a bouw 1.75 acre):

Year:	100 POJ	247 B	EK 28	DI 52	2878 POJ
1912	123	124		148	
1913	**126**	**125**		**167**	
1914	119	115	170	91	
1915	108	105	146	135	
1916	**127**	124	**166**	151	
1917	**142**	**135**	**171**	**152**	
1918	**135**	**127**	158	145	
1919	109	106	133	129	
1920	112	105	134	130	
1921	122	111	139	138	
1922	**126**	116	144	144	
1923	121	111	142	138	
1924	124	115	144	148	
1925		**131**	158	**167**	
1926		106	133	139	167
1927			154	**154**	181
1928			**169**	**177**	190

Among the publications of the Congress is a most noteworthy paper by Prinsen Geerligs, giving a very clear outline of the senility idea. In it he shows that the theory consistently applied leads to conclusions that are contradicted by facts.

Experimental Work on Degeneration.

Many of the experiments on "senility" are based on the research of Schuster, who studied the finer venation of leaves. He distinguishes four types of venation, and measures the density in mm. on a square mm. Sometimes species of one genus belong to different venation types (*Syringa*), more often the densities show great differences as in the genus *Ribes*, where *R.rubrum* has a density of 13.2 and *R.aureum* of 5.2. Comparisons have been made by Schuster between the venation of leaves growing in light and shadow and between the venation of leaves of cuttings and those of the original plant. In the latter case venation showed considerable variation ; sometimes the density was less in cuttings than in the old plant, e.g., in *Achyranthes Verschaffeltii, Laurus nobilis* and *Pelargonium zonale*. But in *Hedera helix* and *Aucuba japonica* the venation was much denser in the leaves of the cuttings.

Benedict was the second authority to investigate venation. He published a preliminary report on his work in 1912, while a longer article appeared in 1915. He compared the average areas enclosed by the smallest veins in old and young plants of the same species. These vein islets appeared to be smaller in old plants than in young ones. The figures for plants of different age are graphed out, and the resulting curve shows considerable analogy with the curve of senility in guinea pigs. He concludes that senility exists in plants, though this is more than his method and material warrant for the following reasons:

1. A decrease of vein-islets *with increasing age* has not been found, only smaller islets in older plants.
2. The correlation between age and area of vein-islets in *Vitis* is very high:—0.90 ± 0.05. This seems an abnormally high index in material which is probably far from homogeneous.
3. The analogy of the curves gives only circumstantial evidence for the identity of the processes which cause them ; moreover the similarity is not so marked that any real analogy can be claimed.
4. The choice of the material does not guarantee homogeneity ; differences due to hereditary factors are probable.
5. Even if increased density of venation with increasing age has been shown, the connection of this phenomenon with senility remains to be proved.

Shortly afterwards Miss Pallis studied the reed (*Phragmites*) lands in the delta of the Danube. The reed occurs in two forms, stout and slender. The river bed contains only the stout form, while the floating mats (Plav), isolated from the rest, consist of slender plants. Miss Pallis considers the slender plants as senile individuals. If this were true, slender reeds ought to be found in other parts of the area too, where old and young plants are to be expected. As slender reeds occur *only* on the floating mats, and not in the large areas nearer the shore, it is more probable that soil conditions are the cause, and that this has not been analyzed by the author.

Neger reports his inability to corroborate Benedict's results, but he does not publish methods or figures.

Ensign has studied seedlings of *Citrus grandis*, originating from sexual reproduction and through apogamous seed-formation, which is, strictly speaking, a form of vegetative propagation. These two classes show no differences in venation, though their " ages " differ. Ensign allows for the possibility that the difference in age was too small to show any marked effect.

Küster reviews Benedict's publication ; he calls the analogy of the two curves unconvincing, but is impressed with the value of the vein-islets study.

Ducomet reports having done research similar to Benedict's without coming to similar results. Description of methods and figures are wanting.

In his later research Ensign followed Benedict's method with the same material in the same institution. He could not, however, find any differences in density of venation between old and young plants, nor a correlation between age and size of the vein islets in six woody plants, among which was *Vitis vulpina*, the principal subject of Benedict's work.

Priestley studied the number of cork layers which are formed on the wounded surface when potatoes are cut in halves. Great differences existed between a number of varieties, but no conclusions could be drawn with reference to the problem of senility. The experiment is being continued in connection with resistance to diseases which enter through the tubers.

Other experiments of the same author, comprising a comparison of growth in seedlings and cuttings, do not open any new vistas on the degeneration problem, owing to the heterogeneity of the seedling material. Interesting though the investigations are, they do not seem to form a basis for further research on " senility."

Tellefsen compared cell-measurements in various tissues in three groups of trees of different ages, taking the diameter of the trunk as an index of age. In comparing the figures she does not make use of mathematical methods. Like her teacher Benedict she draws the incorrect conclusion that dimensions are found to change with increasing age, and does not consider the possibility of hereditary differences in the material. An application of statistical method to the figures contained in her publication affords an entirely different result.

There is no tissue in which the cell dimensions differ between all three age-groups ; only in cross sections of the phlœm are differences to be found in two out of the three comparisons. Three tissues show differences in one out of the three cases, while in the three other tissues the figures for the three age-groups are similar. The correlation between diameter of trunk and cell area have been computed ; only in one case, in longitudinal sections of the cortex, is a marked negative correlation to be found. There is, therefore, very little to support Tellefsen's conclusions.

In the same author's research on vein-islets the difference between the age-groups are smaller than or only as large as the unit of measurement, which definitely makes them of doubtful value.

The Italian Bergamaschi (1926) applied Tellefsen's method to sections of the roots in different plants. Mention is made in her article of differences in cell dimensions between the roots of cuttings and those of seedlings, and the conclusion is reached that senility exists, assuming, of course, that cuttings will be identical in cell dimensions with the parent plant. Applying the statistical method to her figures, differences can be found only in four out of seven cases. The results have to be accepted with reserve, because in general only six observations were made ; in the only case where this number is larger (9), no difference can be detected. The tables, giving statistical results of Tellefsen's and Bergamaschi's publications, which are omitted from this present article, can be found in my longer article on the subject.

Finardi studied venation of trees, counting the number of vein-islets on a given area. Only the averages have been published, rendering it impossible to apply more exact methods to the material. Young and old trees seem to show a marked difference in venation in eight cases ; a small difference can be found in *Tilia parvifolia*. In *Prunus Mahaleb*, of which many figures are given, the differences in venation between a tree of three to four years and one many centuries old appear to be negligible in comparison with the variation among the leaves of a single tree. Generalizing, the writer concludes that the size of the vein-islets diminishes with increasing age, a conclusion which cannot be drawn from the material available.

This list of pertinent literature may be concluded by reference to the observations of Van der Lek, Reed and Molisch (1929) on venation measurements.

The first authority suggests that the wide meshes of the venation may occur only in the juvenile stage of a plant, which will fairly soon reach the narrow mesh stage. He doubts whether the study of venation will ever enable us to draw conclusions on degeneration and senility. This point of view is the same as that of Doflein, 1919, and Ducomet, 1921. Van der Lek emphasizes furthermore the necessity for applying exact methods to the figures given by Bergamaschi.

The American, Reed, remarks in the year 1927, with reference to the venation investigations: "There have been investigations which produced evidence that the size of vein-islets tends to decrease with the age of the individual. The problem is rather difficult, because of the inherent variability of the material, and other investigators have come to the conclusion that there is no difference in the size of the so-called islets. We must therefore conclude that the question is still open."

Molisch, however, in his book on the duration of plant-life (Die Lebensdauer der Pflanze, 1929) recommends the study of Benedict's publications and disapproves of the lack of appreciation they have found. "The investigations show once more, that in the case of asexual propagation . . . the characters of the original plant (in Benedict's investigations the dimensions of the vein-islets) are taken over unchanged by the asexual descendants" (Molisch, p. 145).

Clearly Molisch, like his compatriot Graebner, is still convinced of the existence of senile degeneration.

DUTCH EXPERIMENTS.

In the Laboratorium voor Tuinbouwplantenteelt (Department of Horticultural Research, Prof. A. M. Sprenger) of the Agricultural College at Wageningen, Holland, experiments are in progress by Koopman, Koeman and Bijhouwer. They are not yet in the final stage, but they may serve to orientate further research on venation and as a basis for comparison for many years.

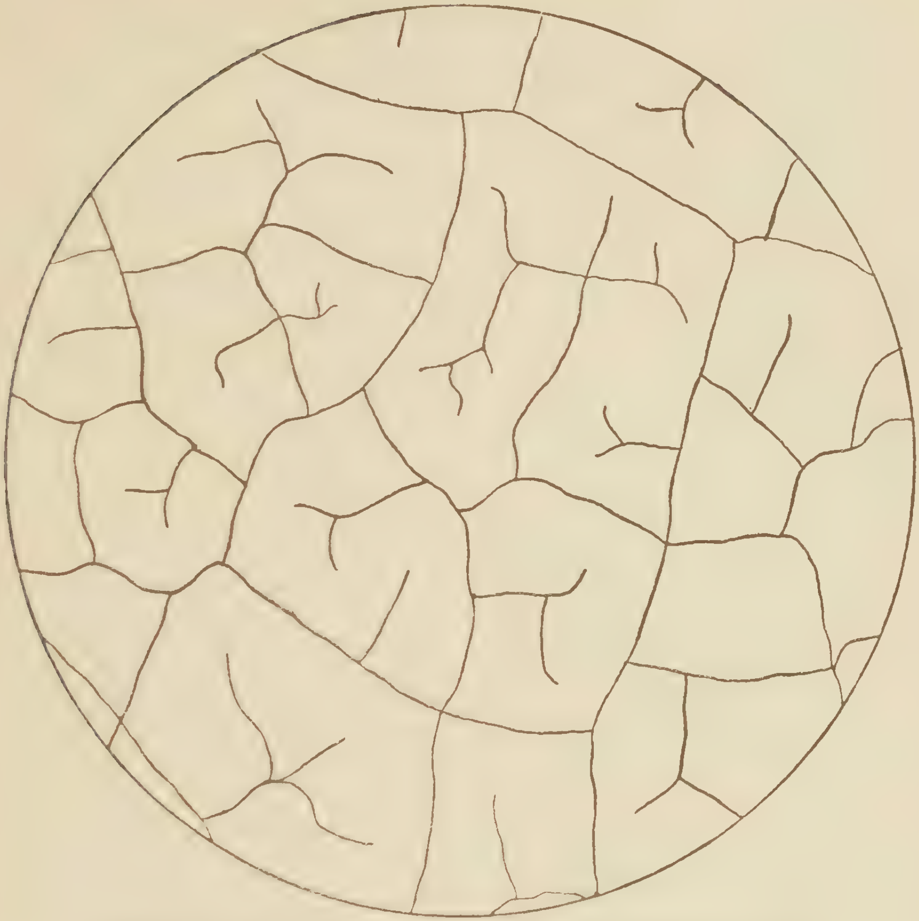
Koopman tested methods of research and found that the following precautions in gathering material are necessary:

1. Plants must be grown as far as possible under identical conditions.
2. Leaves must be taken from a definite part of the twig; lower and upper leaves show considerable differences.
3. Apex and base of leaf must be eliminated in measuring the venation.

Koeman measured the venation of a number of black currant shrubs during the years 1922 and 1923. During the years 1926-1929 the writer of this article continued the experiment. Cuttings were taken from two plants which Koeman had studied and their venation was compared. No definite conclusions have been drawn, as the experiments are still in progress at the same institution, but as preliminary results may be mentioned:

1. *Ribes nigrum* (variety Hoogendijk's Seedling) appears to vary in venation between a density of 7 mm. vein on a sq.mm. and 10 mm./sq.mm. in the material investigated, which is intermediate between *Ribes aureum* (5.2) and *Ribes rubrum* (13.2), (Schuster).
2. The type of venation does not agree fully with one of the four types given by Schuster; usually the "fields" contain one or more branching veinlets, the main branch, or the main branch and some side-branches of which reach the other side of the field.
3. In some cases the venation of cuttings agreed very well with that of the parent plant, but great differences were found between spring cuttings and autumn cuttings of any one plant. The autumn cuttings possess a greater density, the reason of which is still uncertain.

The illustration shows the characteristic venation found in the black currants investigated.



Characteristic venation of the Black Currant, Hoogendijk's Seedling
(8.5 mm. per sq. mm.)

FINAL REMARKS.

The long controversy and all the experiments involved therein have alike failed to solve the problem of senility and have merely given rise to new questions.

If further research is done, the methods and basis of both venation and cell-size study will need investigation ; Schuster's work was not sufficient as a foundation for the series of experiments discussed, since investigation is necessary not only on the changes occurring during the life of the individual together with the variation within one individual, but also on the changes due to asexual and sexual propagation and the variation within species and clone.

For the interpretation of numerical results the use of a mathematical method is absolutely essential.

The cases of " degeneration " which have been discussed (except in the case of *Elodea*, which requires further investigation) have all been interpreted during the last decades as due to disease, to adverse conditions or faulty methods of cultivation. It is thus rendered quite open to question whether in working with clone material any attention need be paid to the theory of senility.

And thus no fruitgrower need fear that his old beloved fruit varieties will die out because of senility. Although throughout the controversy instances have often been cited of " degenerated " clones, improved methods of cultivation or better knowledge of the plant and its shortcomings have in nearly every case shown that " senility " was not the right explanation. Experiments, moreover, have failed to supply convincing proofs.

This article on senility is, of course, but a bird's eye view, a general outline of the problem and a review of the most important relevant publications and their conclusions. If it has been at all helpful to the reader who wishes to form his own opinion on the subject, it has achieved its purpose. For a further report on the experiments in Wageningen and elsewhere, I would refer the reader to the publication which has already been mentioned:

A. P. C. Bijhouwer. Over het vraagstuk der " Veroudering " ten gevolge van langdurig voortgezette, ongeslachtelyke vermeerdering. With a summary in English.

In: Mededeeling No. 12 van het Lab. v. Tuinbouwplantenteelt (Med. Landbouwhoogeschool Dl.34, 1930).

Publishers: H. Veenman & Zonen, Wageningen, Holland.

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(*Received* 26/1/31.)

BOOK REVIEWS.

THE CULTURE OF THE ORANGE AND ALLIED FRUITS. By H. Clark Powell. (Central News Agency, Ltd., South Africa, 1930. £1 1s.)

Professor Clark Powell has produced a book which should earn the gratitude of South African orange growers. While the works of Coit in California and Hume in Florida deal with citrus culture as adapted to the climates of the North American citrus belt, South Africa still lacked a writer who could deal authoritatively from a South African standpoint, and in the light of the latest scientific knowledge, with the innumerable problems, many of them peculiar to the country, with which the citrus grower has to contend.

Every aspect of citriculture from the first selection of the land to the final marketing of the fruit is dealt with in a clear and simple manner.

The question of rootstocks and their effect on the future habit and productivity of the tree is explained in detail. Although vegetative reproduction of rootstocks is not considered commercially feasible by means of cuttings or layers, the author states it is possible by selecting only the strongest seedlings, discarding entirely all others, to ensure that the future yield will be very much greater than would have been the case had the seedling rootstocks been used without discrimination.

This advice is interesting in view of recent discoveries in the polyembryony of the orange, and if conscientiously followed, might well result, after all, in fairly complete stands of vegetatively raised citrus stocks. It will be remembered that Webber and Frost in California and Toxopeus in Java claim that as many as ninety per cent. of seedling oranges are of vegetative origin. If, then, Clark Powell's recommendation is followed and at least fifty per cent. of the young seedling stocks are destroyed, selecting, of course, those that do not conform to standard, it is not too much to argue that among those fifty per cent. will be found the majority of the ten per cent. of generative origin, since these are the more likely to deviate from the main and so to catch the eye of the selector.

The importance of bud selection is stressed, a great proportion of the trees in South Africa having been propagated from inferior bud material, resulting in an enormous number of worthless trees.

There is an account of the planning and analysis of a hypothetical experiment on the Latin Square method of plot lay out. One is however somewhat surprised to find the author, who is rightly insistent on the enormous variation in citrus groves, and who recommends even the ordinary grower to keep some sort of individual tree records (p. 96), recommending the use of eight-tree plots for his own experiment. He says that single tree plots " would entail an enormous amount of detailed management." So they would : but surely no trouble should be too great in an experiment and it is only by such means that an experiment with such variable material can be of the slightest use.

After dealing thoroughly with the problems of marketing, storage, and transport, the book concludes with a complete survey of the industry in the other citrus growing countries. The West Indies, Palestine, Florida and California, are each given a separate chapter. The author, having recently completed a tour of the citrus growing countries for the Empire Marketing Board, is well qualified to speak, and these chapters form by no means the least interesting part of the book.

The illustrations are numerous and clear.

PROCEEDINGS OF THE FIRST IMPERIAL HORTICULTURAL CONFERENCE, London, August, 1930. Imperial Bureau of Fruit Production, East Malling.

Part 1. Papers on the administrative and economic side of horticulture. pp. 36. 1s.
Part 2. Papers on the application of science to horticulture. pp. 58. 2s.
Part 3. Papers on the progress of fruit storage methods. pp. 101, 31 figs. 2s. 6d.

Part 1. This contains a very short summary of work done by the Bureau, the resolutions adopted by the Conference, an economic review of fruit growing in the Empire and an account of the New Zealand Fruit Control Board. The organization of research is dealt with in the remaining papers with special reference to Canada, Australia, Bombay, South Africa, the I.C.I. Station at Jealott's Hill, and a final note on the training of future horticultural research workers is included.

Part 2. General principles are first discussed, after which a section is devoted to the lay-out of field experiments. Certain problems confronting the cider maker are dealt with and some of the chief physiological problems of the horticulturist considered. The last section is devoted to meteorological and soil influences.

Part 3. Papers are given by thirteen of the leading experts in fruit storage in Great Britain, Canada, South Africa, Australia and The West Indies, and deal with the different problems under investigation by them. Most of the papers are illustrated, and include bibliographies. The whole forms a compact symposium, which should prove of great value as a reference book to the research worker.

FIELD EXPERIMENTS IN HORTICULTURE. By T. N. Hoblyn. Imperial Bureau of Fruit Production, East Malling. pp. 50, 16 figs., bibl. 113. 2s.

This is an exposition of the ways in which Dr. R. A. Fisher's statistical methods may be adapted to the difficulties peculiar to fruit-growing.

The author deals with horticultural experiments in the past, details the advantages and disadvantages of the statistical method, and adds notes on special difficulties peculiar to work in the Tropics.

A chapter is devoted to the technique of field experiments with particular reference to deciduous fruits.

Examples are given of three modern horticultural field trials, viz., black currant variety trial, strawberry strain and time of planting trial, apple manurial trial and method of working out the experimental data shown.

The illustrations, diagrams and tables add greatly to the value of the work which concludes with an extensive bibliography.

VEGETABLES OF THE DUTCH EAST INDIES. By J. J. OCHSE, in collaboration with R. C. BAKHUIZEN VAN DEN BRINK. (English edition of Indische Groenten). Department of Agriculture, Industry and Commerce of the Netherland East Indies.

A thought that occurs on first sight of this impressive tome (weighing five and a half pounds and containing over one thousand pages) is that the vegetarian in the Dutch East Indies should have no cause to complain of any lack of variety in his diet. This supposition is confirmed as the book is further examined and the singularity, almost improbability, of some of the comestibles noted. Hevea (rubber) seeds for instance, and the shoots of the castor oil plant, both poisonous in the hands of the unskilled, may appear on the menu in company with such Lucullan delicacies as Damask roses or the " cabbage " heart of a full-grown palm.

In these pages descriptions are given of no less than 389 varieties of edible plants (excluding fruits, which form the subject of another volume), and the author remarks that the subject is far from exhausted. Each description is accompanied by an admirable line drawing by a native artist. The botanical subject matter is arranged alphabetically, first by families, then, still alphabetically within the families, by genera and species.

In dealing with the individual species the botanical name is first given together with the popular name in English, Dutch and three or four native dialects. Then follows a precise botanical description succeeded by notes on cultivation, distribution and use, including as a rule the method of cooking ; finally, authorities are quoted.

Although the author confines himself entirely to plants grown in Java and Madoera, the majority of these plants are to be found throughout the Eastern tropics and it can hardly be doubted that this book is destined to become a standard reference work for those interested in horticulture in the Far East and Northern Australia. The translation into English has been excellently done.

If a fault can be found with the book it is in the too constant use of native words incomprehensible to a non-resident, without any accompanying explanation in English. It is true that there are two substantial glossaries (covering 185 pp.), but continual enforced reference to these, added to some uncertainty as to which of the two will contain the word, engenders in the reader a slight impatience which in reality the book is far from meriting.

FRUIT AND FRUIT CULTURE IN THE DUTCH EAST INDIES. By J. J. OCHSE, in collaboration with R. C. BAKHUIZEN VAN DEN BRINK. (English edition of Vruchten en Vruchtenteelt in Nederlandsch-Oost-Indie). Pub. G. Kolff, Batavia, 1931.

This book purports to be, in the matter of fact words of its author, " a survey of the best and most popular fruits furnished by the Dutch East Indies", but to many a one now shivering in the grip of an English summer it will be a friendly djinn who will transport him, if only in mind, to sun-bathed Hesperidean isles, where all is languorous warmth and flaunting growth, and where fantastic fruits, dangling seductively from the boughs of unfamiliar trees, affright the eyes of orthodox pomologists.

The book deals with fifty-seven species of tropical fruits, each illustrated by a very excellent coloured plate drawn by a Javanese artist. These plates are of especial interest since they depict, besides the fruits known to every traveller, a number of species which are less frequently met with, being more appreciated by the native than by the European. It is probably safe to say that accurate coloured illustrations of such a variety of tropical fruits have not previously been brought together in one handbook. If a criticism may be ventured, it is to regret that, by a stroke of the adverse fortune which too often assails the beautiful and good, that most exquisite fruit the Mangosteen should be endowed with one of the least successful plates.

The plan of the book follows that of its companion volume, " Vegetables of the Dutch East Indies." Following the botanical name come the Dutch, English, French and German equivalents, to be in turn succeeded by a host of native names (banana leading with 154, mango lying a useful second with 122). Then in turn come a full botanical description and sections on the propagation, cultivation, use, general observations, pests and diseases.

The Javanese, although their methods are still primitive, are natural gardeners. Nearly every native dwelling has its " courtyard " averaging about a third of an acre, and these courtyards, besides containing the inevitable coconut and kapok, are packed with fruit trees and other useful plants in a range of varieties of which, the author states, the outsider can form no idea. Since the export of copra and kapok, almost all from trees grown in native yards, together amounts to nearly six million pounds, while vast quantities of miscellaneous fruits are sold in the towns or consumed locally, it would seem that not only does the native household budget derive considerable benefit from this universal fruit production, but also that it must play an important part in the economic life of the country.

The Dutch are doing all in their power to foster this propensity for yard fruit growing and to impress the native with the advantages of scientific culture by modern methods.

This volume should certainly find a place on the shelves of every pomologist in tropical climes, and should be the means of inspiring many an intensive campaign for the establishment or revival of native fruit culture in regions where these fruits would grow freely and materially add to the comfort of the local inhabitants.

BRITISH ASSOCIATION.

CENTENARY MEETING. LONDON, 23RD TO 30TH SEPTEMBER, 1931.

Section M : Agriculture. *Sub-Section :* Horticulture.

A horticultural sub-section of Section M (Agriculture) has been formed. Sir A. D. Hall has consented to act as unofficial head of this sub-section. Meetings have been arranged as follows :—

		Contributors.
Thursday, September 24th 10 a.m.—1 p.m.	Plant Breeding	Sir A. D. Hall. M. B. Crane. W. J. C. Lawrence. Miss C. O'Connor.
Friday, September 25th 10 a.m.—1 p.m.	Vegetative Propagation	Prof. J. H. Priestley. L. B. Stewart. Dr. T. Swarbrick. R. G. Hatton.
Monday, September 28th 10 a.m.—1 p.m.	Glasshouse Problems	Dr. W. F. Bewley. O. Owen. B. D. Bolas. N. L. Hudson.
Tuesday, September 29th 10 a.m.—1 p.m.	Fruit Storage Problems	Dr. F. Kidd. Dr. A. Horne. T. Wallace. Dr. C. West.

On Saturday, September 26th, a visit has been arranged to the East Malling Research Station, by kind invitation of Mr. R. G. Hatton and his staff.

Mr. George Shawyer's much appreciated invitation to visit Messrs. Lowe and Shawyer's Nurseries on Monday afternoon, September 28th, has been accepted.

The Council of the Royal Horticultural Society is most generously affording free admission to the R.H.S. Autumn Shows on September 23rd, 24th, 30th, and on October 1st, to duly accredited members of the British Association.

Particulars of membership of the British Association, temporary and permanent, may be obtained from : The Secretary, British Association for the Advancement of Science, Burlington House, London, W.1.

PRECISION RECORDS IN HORTICULTURE.

By J. O. IRWIN, M.A., D.Sc.

(From Statistical Dept. Rothamsted Experimental Station.)

INTRODUCTION.

The present study is based on observations made on apple trees planted in connection with the Ministry of Agriculture's Horticultural-Meteorological Scheme. This scheme had as its object the study of the relation between weather and the growth of horticultural crops.

A meteorological station, where one did not already exist, was set up in every station participating in the scheme and observations on apples, plums, black currants and peas were made in each place. The observations were started in 1925 and are still continuing. It was laid down that the varieties used were to be the same in each place and a standardised programme of observations was drawn up.

Here we are only concerned with apples. The original scheme laid down that four " maiden " bush trees of both Bramley's Seedling and Worcester Pearmain should be planted on Doucin (Type II.) stock. By 1928 there were actually eight stations (Gulval, Wisbech, Long Ashton, East Malling, Wye, Cambridge, Osgodby and Houghall) at which these varieties were being observed. But three stations (Gulval, East Malling and Osgodby) used temporary substitutes for the observations until the new trees were old enough to observe. In 1926 and 1927 at Gulval and in 1926 at East Malling both temporary substitutes and the new trees were observed. In these cases the new trees were used for measurements of growth (length of three principal leaders taken monthly) and the substitutes for the other observations. At Newton Abbott, Wisley, Perdiswell and Sutton Bonnington comparable trees were not observed. Thus up to 1928 no very large body of comparable data had been obtained. However, the leader measurements at East Malling, Long Ashton, Osgodby and Houghall in the years 1926, 1927 and 1928 were comparable and have been used for the study of growth rates which forms Part I. of this paper.

In addition to the leader measurements the following observations were also to be taken:

(1) Date of (*a*) First open flower.
    (*b*) Full blossom (50 per cent. of flowers in blossom).
    (*c*) Dropping of petals of last flower.

(2) Observer's note on character and duration of blooming season.

(3) Number of blossom trusses (or general observation on quantity of blossom).

(4) General observation on extent of set of fruit.

(5) Time of drop.

(6) Date when bulk of the fruit is ripe.

(7) (*a*) Crop from each tree and total crop gathered, not including June drop, with note of accidental loss.
    (*b*) Total weight of droppings, not including June drop with note of accidental loss.

(8) Note on keeping quantities of fruit.

(9) Diseases and pests (character, date of appearance etc.).

(10) Duration of defoliation period and colour of foliage during this period.

The data were not examined statistically until 1929, when it was found that many of the observations were not so precise and comparable as between different observers working in different places as to make it likely that, even if continued for many years, they would be of great value for correlation with weather phenomena.

For example, an estimate, based merely on inspection of a tree, can determine the 50 per cent. point of flowering neither accurately nor in a manner comparable between different observers in different places.

It appeared that the difficulty could be met by sampling observations of a precise type, accordingly a "*precision-record*" experiment for flowering was carried out on the eight apple trees on the "Horticultural Scheme" plot at East Malling.

This experiment, which was principally concerned with time of flowering, is discussed in Part II. of this paper. Although the determination of dates of flowering in a precise and comparable manner was its main object, it is to be noted that the same principles could be used to get precise and comparable observations on many of the other phenomena which the scheme was designed to study, for example fruit set, June drop, and defoliation and perhaps certain diseases such as apple scab.

PART I.

ON THE GROWTH RATES OF APPLE TREES.

Since 1925 all the horticultural stations which are carrying out the Ministry of Agriculture's Horticultural Scheme, have been taking monthly measurements of three principal leaders on four trees of each of two varieties of apple-trees, Bramley's Seedling and Worcester Pearmain.

The data at four stations, Long Ashton, East Malling, Osgodby and Houghall, in the seasons 1926, 1927 and 1928 are reasonably comparable and may be used for a study of growth rates.

All the trees were three years old in 1926 and on Malling Type II. stock.

The following table shows the units in which the measurements were taken and the degree of accuracy.

TABLE I.

	Long Ashton.	East Malling.	Osgodby.	Houghall.
1926	nearest $\frac{1}{2}$ centimetre.	nearest $\frac{1}{2}$ centimetre.	nearest $\frac{1}{4}$ in.	*May* nearest $\frac{1}{4}$ in. *June* nearest $\frac{1}{2}$ in. rest nearest 1 in.
1927	,,	,,	nearest $\frac{1}{2}$ in.	nearest 1 in.
1928	nearest $\frac{1}{10}$ centimetre.	,,	,,	June nearest $\frac{1}{2}$ in. rest nearest 1 in.

Where measurements were made in inches, they were afterwards converted by the Ministry into centimetres, in 1926 to the nearest half-centimetre and in 1927 and 1928 to the nearest tenth-centimetre.

It is unfortunate that a uniform method of measurement was not adopted, this will be remedied in any future work done ; measurements to the nearest centimetre would be a reasonable level of accuracy.

Using the measurements as given in centimetres, growth rates were calculated on each leader for the months of May, June, July, August and September and relative growth rates for the months of July, August and September. These growth rates were then analysed by the analysis of variance method.*

In each month we have 288 growth rates ; from three leaders on each of four trees of each of two varieties in each of four stations in each of three seasons.

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* The analysis of variance method is one which is being increasingly used by statisticians as, when appropriately handled, it is a very powerful weapon. It has been shown that when we have a series of observations which may be classified into several categories (the number of subdivisions of one category being the same for all the subdivisions of the others) the sum of the squares of the deviations of all the observations from the mean ($S(x-x)^2$) may be expressed as the sum of the number of portions. These portions can be regarded as due to differences within the individual categories and to interactions between these differences. By an interaction between two categories is meant that the differences within any one category vary as we proceed from one subdivision to another of the second category. Thus in the present instance the sum of the squares of the deviations of the 288 observations from their mean can be split up into portions due respectively to seasonal, local and varietal differences and the interactions between them. Now it can be shown that if our data were homogeneous, that is to say if there were really no seasonal, local or varietal differences at all, on dividing each of the portions into which $S(x-x)^2$ has been split up by an appropriate

We thus have 287 degrees of freedom. There are 24 groups of four trees, so that we have 23 degrees of freedom for differences between groups and, since there are 12 growth rates in each group, 264 (11 × 24) degrees of freedom within groups. These 264 may be subdivided into 72 (3 × 24) for differences between trees in the same group and 192 (96 × 2) for differences between leaders on the same tree. This follows because there are 32 trees observed in each of three seasons (3 × 32 = 96) and three leaders measured on each tree. The 23 degrees of freedom between groups can of course be subdivided into portions due to seasonal, place, and varietal differences and their interactions. In fact the complete allocation of degrees of freedom is as follows:

Differences due to:	*Degrees of Freedom.*	
Season	2	
Place	3	
Variety	1	
Interaction between		
Variety and Season	2	
Variety and Place	3	
Place and Season	6	
Second Order Interaction		
Variety, Place and Season	6	
	23	
Trees in Same Group of 4	72	Estimate of Error.
Leaders on Same Tree	192	
	287	

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number known as the *degrees of freedom* we should get an estimate of the same *variance*, namely the total variance of our 288 observations. There estimates would only differ owing to experimental error or kindred sampling fluctuations.

In actual practice we find a course that on dividing say the sum of squares due to season by its *degrees of freedom* we get a larger quantity than that which arises from trees which belong to the same group of four. If this is significantly larger, and a precise test (known as the " *z* " test) exists for determining whether it *is* significantly larger or not, then we know that season is having a significant effect—the same applies to the other categories and to the interactions between them.

The *degrees of freedom* for any one portion of $S(x—x)^2$ can be shown to be equal to the number of *independent* squares into which that portion can be divided.

The *degrees of freedom* of the separate portions add up to the degrees of freedom corresponding to the whole, a number which is one less than the total number of observations.

In practice the analysis is performed by first assigning to each portion the right number of degrees of freedom. This fixes the form of the analysis which can then easily be performed.

The 264 degrees of freedom within groups can be used for our estimate of error.

Tables II. and III. show the results of the analyses of variance for the growth rates and relative growth rates.*

The magnitude of the variances due to season, place variety and their interactions may be compared with that due to leaders which belong to the same group of 4 trees—the degrees of freedom corresponding to these latter are the 264 which have been bracketed together under " estimate of error."

If any of the former variances are significantly larger than the latter then they show up a real effect. The significance is tested by what is known as the " *z* " test. If " *z* " $= \frac{1}{2} \log \frac{v_1}{v_2}$ where v_1, v_2, are two independent estimates of variance " *z* " will be zero if v_1 and v_2 are exactly equal, but fluctuations of sampling will in any case make it different from zero.

Now R. A. Fisher† has tabulated the 5 per cent. and 1 per cent. values of " *z* " such that fluctuations of sampling would only give rise to a large or larger values respectively, once in twenty and once in a hundred times. These values depend on n_1 and n_2 the number of degrees of freedom on which the estimates v_1 and v_2 are based. Here the less stringent 5 per cent. test has been adopted as a criterion of significance. For instance, to test the significance of variance due to season, we work out half the natural logarithm of its ratio to the variance due to error, this gives "*z*." We then enter Fisher's table with $n_1 = 2$, $n_2 = 264$ and determine the 5 per cent. value of " *z*." If this is exceeded by the value above determined, the effect of season is regarded as significant. In Tables II. and III. those portions of the variance which are not significant as judged by the 5 per cent. " *z* " test are put in brackets.

Tables IV.-XI. give the mean values and appropriate standard errors in each month, year and place for each variety.

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* The growth rate in cms. per day for any one leader is obtained by dividing the growth of the leader during the month, measured in cms., by the number of days in the month.

The relative growth rate is the proportional amount by which the growth increases in the unit of time. Thus if U_0 be the length of a leader at the beginning of a month of thirty-one days, U_1 at the end of the month, α the relative growth rate per day is given by

$$U_0 \; (1+\alpha)^{31} = U_1$$

$$\text{or } \log_e \; (1+\alpha) = \tfrac{1}{31} \; (\log_e U_1 - \log_e U_0)$$

or sufficiently approximately

$$\alpha = \tfrac{1}{31} \; (\log_e U_1 - \log_e U_0).$$

Thus the relative growth rate can be conveniently calculated by subtracting the natural logarithms of the leader length at the beginning from that at the end of the month, and dividing by the number of days in the month. The result can then be multiplied by 100 to express it as a percentage per day.

† Statistical methods for Research Workers—Appendix.

TABLE II.

Growth Rates—Apples (cms. per day)—Analyses of Variance.

		MAY.		JUNE.		JULY.		AUGUST.		SEPTEMBER.	
Due to	D.F.	S. of S.	Mean Square.	S. of S.	Mean Square.	S. of S.	Mean Square.	S. of S.	Mean Square.	S. of S.	Mean Square.
Season	2	.088	.044	.539	.270	.013	(.007)	.720	.360	.359	.180
Place	3	3.353	1.118	2.325	.775	4.276	1.425	2.478	.826	.507	.169
Variety	1	.262	.262	.039	(.039)	.001	(.001)	.088	.088	.039	.039
Interaction between : Variety and Season ..	2	.408	.204	.231	.116	.256	.128	.182	.091	.038	(.019)
Variety and Place ..	3	.049	.016	.078	(.026)	.085	(.028)	.228	.076	.042	(.014)
Place and Season ..	6	1.115	.186	3.695	.616	1.415	.236	.731	.122	.280	.047
Second Order Interaction : Variety, Place and Season ..	6	.165	.027	.326	.054	.169	(.028)	.403	.067	.076	(.013)
Trees in same group of 4 .. Leaders on same tree ..	72 192	.633 .818	.009 } .005 .004 }	2.660 3.596	.037 } .024 .019 }	5.148 4.256	.072 } .036 .022 }	2.749 2.888	.038 } .021 .015 }	.688 1.189	.010 } .007 .006 }
Total	287	6.891		13.489		15.619		10.467		3.218	

D.F. = Degrees of Freedom.

S. of S. = Sum of Squares.

Figures in brackets are not significant on the basis of the 5% "*z*" test

TABLE III.

Relative Growth Rates—Apples (% per day)—Analyses of Variance.

Due to	D.F.	JULY.		AUGUST.		SEPTEMBER.	
		S. of S.	Mean Square.	S. of S.	Mean Square.	S. of S.	Mean Square.
Season	2	.361	(.181)	2.775	1.388	1.869	.935
Place	3	108.653	36.218	12.656	4.219	2.843	.948
Variety	1	1.468	(1.468)	.118	(.118)	.040	(.040)
Interaction between : Variety and Season	2	3.472	1.736	.981	.491	.073	(.037)
Variety and Place	3	.428	(.143)	1.404	.468	.170	(.057)
Place and Season	6	35.052	5.842	10.457	1.743	2.603	.434
Second Order Interaction : Variety, Place and Season ..	6	6.300	1.050	2.688	.448	.365	(.061)
Trees in same group of 4 ..	72	48.095	.668 } .475	14.297	.119 } .138	7.020	.098 } .060
Leaders on same tree	192	77.350	.403	22.263	.116	8.789	.046
Total	287	281.179		67.639		23.772	

D.F.=Degrees of Freedom.

S. of S.=Sum of Squares.

Figures in brackets are not significant on the basis of the 5% " z " test.

The results which they show are discussed fully in the next section of the paper, but a word may be interpolated here on the use of the standard errors at the foot of the tables. The standard error is smaller the greater the number of observations on which the average is based (being inversely proportional to the square root of that number). If it is desired to compare two averages based on the same number of observations their difference may be compared with 2.8 or roughly three times the appropriate standard error given below the table.

Thus in Table IV. we find that at Long Ashton in May, 1926, the growth rate of Bramley exceeded that of Worcester by .145, the corresponding standard error is .023, three times this is .069, so that a difference of .145 may be regarded as significant. But if we wished to compare the means of the two varieties at Long Ashton in 1926 and 1927, whose difference is .070, the appropriate standard error is .016. Again the result would be significant. The reason for the smaller standard error is that twice the number of observations have gone to form the average. Other cases may be treated analogously.

If we desire to compare two averages based on different numbers of observations we must compare the difference between the averages with its standard error. The latter is obtained by taking the square root of the sum of the squares of the standard errors of the separate averages. A difference which exceeds twice this may be regarded as significant.

Thus in Table IV., to compare Worcester at Long Ashton in 1926 with the mean at that place for all three years (both varieties), we have $.180 - .162 = .018$.

The standard error of this difference is $\sqrt{(.023)^2 + (0.009)^2} = .025$ and the difference is not significant.

TABLE IV.

Growth Rates. May.

(Average Values—cms. per day per Leader.)

		1926.		1927.		1928.		All Years.	
Long Ashton ..	W	.162	.235	.192	.165	.099	.141	.151	.180
	B	.307		.137		.182		.209	
East Malling ..	W	.231	.277	.555	.477	.285	.374	.357	.376
	B	.322		.400		.463		.395	
Osgodby	W	.009	.082	.224	.238	.165	.234	.133	.185
	B	.154		.252		.303		.236	
Houghall	W	.118	.128	.000	.000	.053	.105	.057	.077
	B	.137		.000		.156		.098	
All Places	W	.130	.180	.243	.220	.151	.213	.175	.205
	B	.230		.197		.276		.234	

		cms. per day.
Standard Errors. For Individual Entries	(one variety)	.023
	(mean of both varieties)	.016
Means of all Places in Individual Years	(one variety)	.012
	(both)	.008
Means of all Seasons in Individual Places	(one variety)	.013
	(both)	.009
General Means	(one variety)	.006
	(both)	.005

TABLE V.

Growth Rates. June.

(Average Values—cms. per day per Leader.)

		1926.		1927.		1928.		All Years.	
Long Ashton	W	.293	.340	.420	.535	.179	.126	.297	.334
	B	.387		.649		.072		.369	
East Malling	W	.467	.437	.422	.481	.442	.441	.444	.453
	B	.408		.539		.440		.462	
Osgodby	W	.444	.422	.613	.651	.712	.666	.590	.580
	B	.399		.688		.619		.569	
Houghall	W	.590	.593	.310	.302	.275	.314	.392	.403
	B	.597		.295		.353		.415	
All Places	W	.449	.448	.441	.492	.402	.387	.431	.443
	B	.448		.543		.371		.454	

		cms. per day.
Standard Errors. For Individual Entries	(one variety)	.014
	(mean of both varieties)	.010
Means of all Places in Individual Years	(one variety)	.007
	(both)	.005
Means of all Seasons in Individual Places	(one variety)	.008
	(both)	.006
General Means	(one variety)	.004
	(both)	.003

TABLE VI.

Growth Rates. July.

(Average Values—cms. per day per Leader.)

		1926.		1927.		1928.		All Years.	
Long Ashton ..	W B	.227 .344	.286	.093 .180	.137	.054 .030	.042	.125 .185	.155
East Malling ..	W B	.137 .268	.202	.395 .260	.327	.390 .302	.346	.307 .277	.292
Osgodby	W B	.381 .411	.396	.380 .403	.392	.601 .497	.549	.454 .437	.446
Houghall	W B	.462 .480	.471	.423 .472	.448	.463 .390	.427	.449 .447	.448
All Places	W B	.302 .376	.339	.323 .329	.326	.377 .305	.341	.334 .337	.335

		cms. per day.
Standard Errors. For Individual Entries	(one variety)	.054
	(mean of both varieties)	.038
Means of all Places in Individual Years	(one variety)	.027
	(both)	.019
Means of all Seasons in Individual Places	(one variety)	.031
	(both)	.022
General Means	(one variety)	.016
	(both)	.011

TABLE VII.

Relative Growth Rates. (% per day.) July.

		1926.		1927.		1928.		All Years.	
Long Ashton ..	W B	1.007 .939	.973	.532 .547	.540	.636 .327	.481	.725 .604	.665
East Malling ..	W B	.520 .887	.703	1.078 .732	.905	1.339 .945	1.142	.979 .855	.917
Osgodby	W B	2.350 1.862	2.106	1.252 1.192	1.222	1.717 1.460	1.589	1.773 1.505	1.639
Houghall	W B	1.521 1.488	1.505	2.418 3.095	2.757	2.828 2.011	2.420	2.256 2.198	2.227
All Places	W B	1.350 1.294	1.320	1.320 1.392	1.320	1.630 1.186	1.408	1.433 1.291	1.362

		%
Standard Errors. For Individual Entries	(one variety)	.199
	(mean of both varieties)	.141
Means of all Places in Individual Years	(one variety)	.099
	(both)	.070
Means of all Seasons in Individual Places	(one variety)	.114
	(both)	.081
General Means	(one variety)	.057
	(both)	.041

TABLE VIII.

Growth Rates. August.

(Average Values—cms. per day per Leader.)

		1926.		1927.		1928.		All Years.	
Long Ashton ..	W	.016	.093	.043	.079	.032	.030	.030	.067
	B	.170		.114		.027		.104	
East Malling ..	W	.093	.077	.182	.179	.010	.055	.095	.104
	B	.061		.177		.101		.113	
Osgodby	W	.285	.428	.338	.358	.157	.140	.260	.309
	B	.572		.378		.122		.357	
Houghall	W	.150	.153	.320	.238	.157	.163	.209	.185
	B	.157		.156		.168		.160	
All Places	W	.136	.188	.221	.214	.089	.097	.149	.166
	B	.240		.206		.105		.184	

		cms. per day.
Standard Errors. For Individual Entries	(one variety)	.042
	(mean of both varieties)	.030
Means of all Places in Individual Years	(one variety)	.021
	(both)	.015
Means of all Seasons in Individual Places	(one variety)	.024
	(both)	.017
General Means	(one variety)	.012
	(both)	.009

TABLE IX.

Relative Growth Rates. (% per day.) August.

		1926.		1927.		1928.		All Years.	
Long Ashton	W B	.062 .382	.222	.155 .347	.251	.383 .286	.335	.200 .338	.269
East Malling	W B	.332 .192	.262	.409 .398	.404	.032 .259	.146	.258 .283	.271
Osgodby	W B	.944 1.518	1.231	.775 .792	.783	.317 .264	.290	.679 .858	.768
Houghall	W B	.367 .385	.376	1.023 .465	.744	.537 .536	.537	.642 .462	.552
All Places	W B	.426 .619	.523	.591 .501	.546	.317 .336	.327	.445 .485	.465

		%
Standard Errors. For Individual Entries	(one variety)	.107
	(mean of both varieties)	.076
Means of all Places in Individual Years	(one variety)	.054
	(both)	.038
Means of all Seasons in Individual Places	(one variety)	.062
	(both)	.044
General Means	(one variety)	.031
	(both)	.022

TABLE X.

Growth Rates. September.

(Average Values—cms. per day per Leader.)

		1926.		1927.		1928.		All Years.	
Long Ashton	W B	.034 .124	.079	.037 .083	.060	.009 .012	.011	.027 .073	.050
East Malling	W B	.099 .061	.080	.000 .000	.000	.000 .000	.000	.033 .020	.027
Osgodby	W B	.166 .143	.155	.137 .271	.204	.007 .030	.018	.103 .148	.126
Houghall	W B	.112 .117	.115	.119 .162	.141	.092 .085	.089	.108 .121	.115
All Places	W B	.103 .111	.107	.073 .129	.101	.027 .032	.029	.068 .091	.079

		cms. per day.
Standard Errors. For Individual Entries	(one variety)	.024
	(mean of both varieties)	.017
Means of all Places in Individual Years	(one variety)	.012
	(both)	.008
Means of all Seasons in Individual Places	(one variety)	.014
	(both)	.010
General Means	(one variety)	.007
	(both)	.005

TABLE XI.

Relative Growth Rates. (% per day.) September.

		1926.		1927.		1928.		All Years.	
Long Ashton ..	W	.118	.194	.110	.158	.082	.100	.103	.151
	B	.269		.207		.118		.198	
East Malling ..	W	.322	.273	.000	.000	.000	.000	.107	.091
	B	.224		.000		.000		.075	
Osgodby	W	.432	.367	.272	.374	.019	.042	.241	.261
	B	.302		.475		.065		.281	
Houghall	W	.219	.244	.564	.549	.273	.254	.352	.349
	B	.268		.534		.234		.345	
All Places	W	.273	.269	.237	.270	.094	.099	.201	.213
	B	.266		.304		.104		.225	

		%
Standard Errors. For Individual Entries	(one variety)	.071
	(mean of both varieties)	.050
Means of all Places in Individual Years	(one variety)	.035
	(both)	.025
Means of all Seasons in Individual Places	(one variety)	.041
	(both)	.029
General Means	(one variety)	.020
	(both)	.014

(i) *Place and Seasonal Differences and their Interactions.*

We can get the clearest view of the meaning of our results, if in each year and month we arrange the four places in order of their growth rates. In the following table E.M. stands for East Malling, L.A. for Long Ashton, O. for Osgodby and H. for Houghall. A mean value is put in the same line as its neighbour when not significantly different from it.

TABLE XII.

Places in Descending Order of Growth Rates.

May. Growth Rates.

1926.	1927.	1928.	All Years.
E.M., L.A.	E.M.	E.M.	E.M.
H	O	O	O, L.A.
O	L.A.	L.A.	H
	H	H	

June. Growth Rates.

1926.	1927.	1928.	All Years.
H	O	O	O
E.M., O	L.A.	E.M.	E.M.
L.A.	E.M.	H	H
	H	L.A.	L.A.

July. Growth Rates.

1926.	1927.	1928.	All Years.
H, O	H, O, E.M.	O	H, O
L.A., E.M.	L.A.	H, E.M.	E.M.
		L.A.	L.A.

July. Relative Growth Rates.

1926.	1927.	1928.	All Years.
O	H	H	H
H	O	O	O
L.A., E.M.	E.M., L.A.	E.M.	E.M.
		L.A.	L.A.

August. Growth Rates.

1926.	1927.	1928.	All Years.
O	O	H, O	O
H, L.A., E.M.	H, E.M.	E.M., L.A.	H
	L.A.		E.M., L.A.

August. Relative Growth Rates.

1926.	1927.	1928.	All Years.
O H, E.M., L.A.	O, H E.M., L.A.	H, L.A., O, E.M.	O H E.M., L.A.

September. Growth Rates.

1926.	1927.	1928.	All Years.
O, H, E.M., L.A.	O H L.A. E.M.	H O, L.A., E.M.	O, H L.A., E.M.

September. Relative Growth Rates.

1926.	1927.	1928.	All Years.
O, E.M., H, L.A.	H O L.A. E.M.	H L.A., O, E.M.	H O L.A., E.M.

Considering first the general order as exhibited in the " All Years " column, we see that in May East Malling shows the greatest growth, as we might expect from its geographical position, Osgodby in Yorkshire and Long Ashton in Somerset are somewhat surprisingly on a level, while Houghall (Durham) lags behind. In June Osgodby has risen to top place, while Long Ashton has sunk to the bottom. In July, whether one looks at growth rates or relative growth rates, Houghall and Osgodby occupy the top position, while Long Ashton is still at the bottom. Thus the two northern stations seem to be making up for the time lost earlier in the season. In August the position of the two northern stations are reversed, otherwise the same order is maintained. In September, apart from the reversal of the two northern stations in the order of relative growth rates, the same position is maintained. It is to be noted that Long Ashton throughout occupies a lower position than one might expect.

A study of the order in the individual seasons brings out the meaning of the significant interaction between variety and place which may be noted in the analysis of variance.

The order of places in respect of the magnitude of the growth rates *does* change significantly from season to season. On the whole it appears that 1927 and 1928 were similar in their effect on this order while 1926 was relatively anomalous. The difference between 1926 and the other two seasons is most marked in May. In May, 1926, Long Ashton headed the list with East Malling ; Houghall was second and Osgodby third.

As regards direct seasonal effect, in the following tables the seasons are shown in descending order of growth rates. Where one year is not significantly different from its neighbour it appears in the same line.

Table XIII.

Seasons in Descending Order of Growth Rates.

	Growth Rates.	Relative Growth Rates.
May	1927, 1928 1926	
June	1927 1926 1928	
July	1928, 1926, 1927	1928, 1927, 1926
August	1927, 1926 1928	1927, 1926 1928
September ..	1926, 1927 1928	1927, 1926 1928

It appears that in May there was significantly less growth in 1926 than in the other two years, in June significantly less in 1926 than in 1927 and still less in 1928, in July no significant differences, while in August and September there was significantly less growth in 1928 than in the other two years.

(ii) *Direct Varietal Differences.*

Taking the average figures of all years and places, the following table shows which has the more growth, Bramley or Worcester.

Table XIV.

Bramley and Worcester Compared.

	Growth Rates.	Relative Growth Rates.
May	B > W	
June	B > W	
July	No difference	No difference
August	B > W	No difference
September ..	B > W	No difference

Thus while there is no difference in relative growth rate, Bramley on the average has the greater absolute amount of growth.

(iii) *Interaction of Season and Variety.*

Nevertheless this is not true in all seasons, as may be gathered from the interaction between season and variety in the analysis of variance, which is significant except in September.

The meaning of this is brought out in the following table.

TABLE XV.

Bramley and Worcester Compared in Different Seasons.

(Average for all places.)

		Growth Rates.	Relative Growth Rates.
May ..	1926	B > W	
	1927	W > B	
	1928	B > W	
June ..	1926	No difference	
	1927	B > W	
	1928	W > B	
July ..	1926	B > W (just below significance line)	No difference
	1927	No difference	No difference
	1928	W > B (just below significance line)	W > B
August ..	1926	B > W	B > W
	1927	W > B	No difference
	1928	No difference	No difference
September	1926	B > W	No difference
	1927	B > W	No difference
	1928	No difference	No difference

Thus we can see that 1926 was more favourable to Bramley and less favourable to Worcester than 1927 or 1928. We have already seen that 1926 was also relatively anomalous in its effect on the order of stations in respect of growth.

(iv) *Interaction of Variety and Place and the Second Order Interaction—Variety, Place and Season.*

Reference to the Analyses of Variance shows that interactions between variety and place were significant for growth rates in May and August, and for relative growth rates in August only. The second order interaction was significant for growth rates in May, June and August and for relative growth rates in July and August. The meaning of this is brought out in the following table, which compares growth of Bramley and Worcester in all years, and in each year separately at each place:

TABLE XVI.

Bramley and Worcester Compared in Different Seasons and Places.

May. Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton ..	B > W	B > W	No difference	B > W
East Malling	B > W	B > W	W > B	B > W
Osgodby	B > W	B > W	No difference	B > W
Houghall	B > W	No difference	No difference	B > W

June. Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton	B > W	B > W	B > W	W > B
East Malling ..	No difference	W > B	B > W	No difference
Osgodby	No difference	W > B	B > W	W > B
Houghall	B > W	No difference	No difference	B > W

July. Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton	No difference	No difference	No difference	No difference
East Malling	No difference	No difference	No difference	No difference
Osgodby	No difference	No difference	No difference	No difference
Houghall	No difference	No difference	No difference	No difference

July. Relative Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton	No difference	No difference	No difference	No difference
East Malling	No difference	No difference	No difference	No difference
Osgodby	No difference	No difference	No difference	No difference
Houghall	No difference	No difference	B > W	W > B

August. Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton	B > W	B > W	No difference	No difference
East Malling	No difference	No difference	No difference	No difference
Osgodby	B > W	B > W	No difference	No difference
Houghall	No difference	No difference	No difference	No difference

August. Relative Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton	No difference	B > W	No difference	No difference
East Malling	No difference	No difference	No difference	No difference
Osgodby	B > W	B > W	No difference	No difference
Houghall	W > B	No difference	W > B	No difference

September. Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton	B > W	B > W	No difference	No difference
East Malling	No difference	No difference	No difference	No difference
Osgodby	B > W	No difference	B > W	No difference
Houghall	No difference	No difference	No difference	No difference

September. Relative Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton ..	No difference	No difference	No difference	No difference
East Malling	No difference	No difference	No difference	No difference
Osgodby	No difference	No difference	B > W	No difference
Houghall	No difference	No difference	No difference	W > B

First as regards interaction between variety and place, turning to the "All Years" column, we see that, in May, Bramley always shows more growth than Worcester. As the interaction is nevertheless significant, the reason must be found in the *varying amounts* by which Bramley's exceed Worcester in the different places. Reference to Table IV. shows that at Long Ashton this amount is .06 cms. per day ; .10 at Osgodby and .04 at East Malling and Houghall.

The other significant case is in August, both for growth-rates and relative growth-rates. Here we find that while the absolute growth of Bramley is greater than that of Worcester at Long Ashton and Osgodby, there is no difference at East Malling and Houghall. · In relative growth, however, there is no difference at Long Ashton and East Malling ; Bramley exceeds Worcester at Osgodby and the reverse is true at Houghall.

The significant second order interaction shows us that the relative behaviour of Bramley and Worcester in the four places varies from season to season. In May, for instance, whereas in 1928 Bramley showed more growth than Worcester in all four places, in 1926 this was only true in three ; and in 1927 Worcester showed more growth than Bramley at East Malling and in the remaining places there was no difference. In June there was again significant differences in relative behaviour, but of a different nature, in August two seasons show no significant differences at all, while in 1926 Bramley showed more growth than Worcester in two stations (Long Ashton and Osgodby).

Turning to relative growth rates in July, we find no differences except in one station (Houghall), in only two seasons (1927 and 1928). In the former case Bramley beats Worcester, in the latter the reverse is true. In August in 1926 we find Bramley beating Worcester in two stations (Long Ashton and Osgodby) and no difference in the other two ; in 1927 there is only a difference at one station (Houghall), this time in favour of Worcester ; in 1928 there are no differences.

The cases where the interactions are not significant may be safely disregarded here.

SUMMARY.

(*a*) Over the period as a whole we find:

(i) East Malling (Kent) gets growth started first showing the greatest growth rates in May, but, as one might expect, loses this advantage later in the season.

(ii) Long Ashton (Somerset) shows rather low growth rate over the whole season.

(iii) Osgodby (Yorks) gets started rather early considering its geographical position, actually equalling Long Ashton in May.

(iv) Houghall (Durham) makes up for its late start by having the greatest growth rates in July.

(v) The absolute amount of growth in Bramley is greater than in Worcester, but there is no difference in the relative growth rates.

(vi) In the May and August growth rates, and in the August relative growth rate, there are significant interactions between variety and place. The advantage Bramley has over Worcester is greater at Long Ashton and Osgodby than at East Malling and Houghall.

(*b*) When we consider seasonal effects we find:

(i) In May there was significantly less growth in 1926 than in the other two years; in June significantly less in 1926 than in 1927 and still less in 1928; in July there were no significant differences, while in August and September there was significantly less growth in 1928 than in the other two years.

(ii) The order of places in respect of the magnitude of the growth rates changes significantly from season to season; 1927 and 1928 were similar in their effect, while 1926 was relatively anomalous. The difference between 1926 and the other two seasons is most marked in May. Here the order for all years is East Malling, Osgodby, Long Ashton and Houghall, while in 1926 the order is Long Ashton, East Malling, Houghall and Osgodby.

(iii) Season also has an influence on the relative behaviour of varieties, 1926 was more favourable to Bramley and less favourable to Worcester than 1927 or 1928.

(iv) The relative behaviour of Bramley and Worcester in the four places also varies significantly from season to season in the months of May, June and August for absolute growth rates, and in July and August for relative growth rates.

(v) To specify the weather factors which are responsible for these somewhat puzzling interactions with season would need data over many more years than are now available.

PART II.

ON TIME OF FLOWERING.

(i) *General Considerations.*

With the object of seeing whether the horticultural observations taken in connection with the Ministry of Agriculture's Crop Weather Scheme could not be improved by observations of a precision-record type such as have been employed successfully in the agricultural portion of the scheme, an experiment on the flowering of apple trees at East Malling was carried out in 1930. The eight trees on the plot which had been laid down in connection with the Horticultural Crop Weather Scheme of the Ministry were used. Four of the trees were Bramley's Seedling and four Worcester Pearmain, all six-year-old trees on Doucin (No. II.) stock.

Four samples of 200 cms. of wood were measured and marked off on each tree. The method of sampling employed was that now generally in use at East

Malling. The tree is approached from the four points of the compass in turn ; each time the eyes are closed and the right hand stretched out to touch a branch. From the point where a branch is touched we follow down until we reach a main branch, and then we follow the main branch up again to its tip. This may of course lead us to a different side of the tree from our starting point. From the tip of the main branch thus reached we start measuring downwards ; as soon as we come to a fork we follow the lateral up to its tip, and continue measuring from the outside inwards ; once back at the fork we continue measuring the main branch until we get to the next fork and so on. In this way any desired length of wood can be measured and we can mark the end of our measured portion with a piece of tape tied round it.

On a mature tree this is as near an approach to random sampling as is practicable ; actually on the six-year-old trees the four samples of 200 cms. of wood took up a very considerable portion of the tree, the main branch reached usually being on the same side of the tree as that touched.

On May 1st the total number of fruit buds on each tree as a whole were counted and the four samples of 200 cms. of wood were marked off.

The number of fruit buds on the sample branches were also counted. The term fruit bud is here used to mean the entity which gives rise to the truss, as distinguished from the individual blossom buds. At this time the fruit buds were just bursting and the individual blossom buds were becoming visible. On May 5th the individual blossom buds on the four samples on each of the eight trees were also counted.

Table I. shows the total number of fruit buds on each of the eight trees and also the number included in the four samples on each tree.

TABLE I.

	Tree.	Total No. of Fruit Buds.	No. of Fruit Buds on Branches Sampled.
Bramley	1	108	38
,, ...	2	81	41
,, ...	3	144	51
,, ...	4	66	34
Total		399	164
Worcester	1	136	99
,, ...	2	136	116
,, ...	3	191	85
,, ...	4	223	81
Total		686	381
Grand Total ..		1,085	545

In the aggregate about half the flower trusses were therefore sampled, the proportion naturally varying from tree to tree.

The total numbers of individual flower buds on the sample branches were as follows:

TABLE II.

Number of Individual Flower Buds on Sample Branches.

Tree	Bramley.	Worcester.*
1	165	435
2	198	511
3	230	340
4	140	296
	733	1,582

Of the 733 Bramley buds only 644 ever opened, the remainder being destroyed before opening, mostly by insect pests. Of the 1,582 Worcester buds 1,546 opened.

On June 17th the fruit set on the sampled branches was counted. Twenty-two out of the thirty-two apples on the Worcesters, but only four out of the forty-eight on Bramley's were found to be attacked by saw-fly. On September 24th the mature fruits on the sampled branches were counted. The Worcesters had none and the Bramley's seventeen fruits.

The full particulars are given in Table III., which shows the percentage of fruit set and of mature fruit obtained. The percentages were calculated both per truss and per individual flower-bud number. In the latter case, had the percentage been calculated on the basis of the number of flowers which opened, the advantage of Bramley as compared with Worcester would have been even more emphasised.

The two varieties form a striking contrast. On the Worcesters there were more than double the number of flowers which were on the Bramley's, yet the latter variety set 50 per cent. more fruit. The percentage of fruit set on the Worcesters was only about a third of that of the Bramley's and finally the former yielded no fruit at all.

(ii) *Daily flowering counts.*

Starting on May 9th, daily counts were made of the number of flowers open on each of the sample branches. The number of flowers, all the petals of which had dropped, were also counted. The original data are shown in Tables

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* These are the numbers of flower buds obtained at the first count, when evidently a few were missed, because the numbers of flowers finally recorded on certain branches (as having flowered) were sometimes slightly greater than the initial number of buds. See Appendix, Tables VI.-IX.

TABLE III.

Bramley's Seedling. Four Samples of 200 cms. of wood.

				Fruit Set.			Mature Fruit.		
Tree No.	No. of Flower Buds.	No. of Trusses.	Flowers per Truss.	No.	% per Truss.	% per Flower Bud.	No.	% per Truss.	% per Flower Bud.
1	165	38	4·3	18	47·4	10·9	6	15·8	3·6
2	198	41	4·8	11	26·8	5·6	3	7·3	1·5
3	230	51	4·5	11	21·6	4·8	3	5·9	1·3
4	140	34	4·1	8	23·5	5·7	5	14·7	3·6
Total	733	164	4·5	48	29·3	6·5	17	10·4	2·3

Worcester Pearmain. Four Samples of 200 cms. of wood.

				Fruit Set.			Mature Fruit.		
Tree No.	No. of Flower Buds.	No. of Trusses.	Flowers per Truss.	No.*	% per Truss.	% per Flower Bud.	No.	% per Truss.	% per Flower Bud.
1	435	99	4·4	5	5·1	1·1	*Nil.*	*Nil.*	*Nil.*
2	511	116	4·4	14	12·1	2·7			
3	340	85	4·0	8	9·4	2·4			
4	296	81	3·7	5	6·2	1·7			
Total	1,582	381	4·2	32	8·4	2·0			

* Tree 1 2 attacked by sawfly.
Tree 2 11 ,, ,,
Tree 3 5 ,, ,,
Tree 4 3 ,, ,,

VI-IX. of the Appendix.* The complete frequency distributions both of time of opening and of time of drop were obtained from these figures.†

The following diagram shows the position of the trees in the plot:

N

	P_4	W_2	
	W_4	B_2	
W	B_4	P_2	E
	P_3	W_1	
	W_3	B_1	
	B_3	P_1	

S

P_1, P_2, P_3, P_4 are plum trees not observed in this connection.

In order to investigate whether the position of the trees had any obvious influence on flowering, the frequencies were added together for the two trees of each variety on the East and on the West sides. Fig. 1 shows the cumulative frequencies, i.e., the number of flowers which had opened by given dates for W_1 and W_2 together, for W_3 and W_4 together, and similarly for the Bramley's of the East and West sides.

It will be seen that the Worcesters on the East side had more flowers than those on the West, but the reverse was true of the Bramley's.

A similar state of affairs is exhibited in Fig. 2, which shows the drop. There is even less difference between the North and South positions, so that there is no evidence here to suggest that the differences are due to the geographical position of the trees on the plot.

It has been remarked that the branches sampled on each tree were roughly in the N, S, E and W positions. Accordingly the number of flowers opening and dropping on the branches in the North, East, South and West positions were obtained for each variety. Fig. 3 shows the cumulative frequencies for opening and Fig. 4 for drop. It will be seen that there is little or no difference between branches in the West and North positions, on the Worcesters the branches on the East side had more flowers than those on the South side, and the reverse was true for the Bramley's. There is therefore no evidence that the position of branches on the tree was a factor of any importance in relation to flowering.

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* On May 20th on B_3 there was difficulty on the West side and on B_4 on the East side, in a few cases indistinguishing those blossoms which had flowered and were over, from those buds which had never opened but the petals of which had become shrivelled and eaten away owing to attacks of insect pests. This continued on the West side on the 21st and 22nd. Accordingly in Table IX. the numbers of flowers dropped are in certain cases marked with a star, to show that they have been estimated, where this was necessary.

† By the "*complete frequency distribution of time of opening*" is meant the data which show for every day in the flowering season how many flowers have opened on that day. The data may conveniently be portrayed by a diagram which represents the number of flowers opening on any day by a block whose height is proportional to that number. Frequency distribution of time of drop has a similar meaning. For examples of such diagrams see Figures 9, 10, 13 and 14.

It may be noted that each Bramley on the plot was next to a Worcester. In Figures 5 and 6 the number of flowers on adjacent pairs of trees are compared. It will be seen that there is very little difference between positions 1 and 3. But position 2 has actually done better than position 4, while 1 and 3 are intermediate. This is remarkable, considering the closeness of the two positions (2 and 4) and may be a soil effect, but we cannot say with only two trees in each position.

(iii) *Detailed study of the frequency distributions for Bramley and Worcester.*

The total frequency distributions for Bramley and Worcester (all four trees together) are shown in Figs. 7-14. Figs. 7-10 concern flowering and Figs. 11-14 concern drop. In Fig. 7 the cumulative frequencies are shown and Fig. 8 the cumulative percentage frequencies. Fig. 9 shows the actual daily frequencies and in Fig. 10 these are expressed as percentages. Figs. 11 to 14 are constructed on the same plan for the drop.

Since the main object of the experiment was to determine date of flowering and drop in a manner comparable between different observers in different places, we naturally consider this first. Date of flowering is most simply defined as the date when half the flowers have come out, and date of drop as the date when half the flowers have dropped. That is to say, we take the medians* of the frequency distributions.

We find the date of flowering was the 15th for Bramley and the 16th for Worcester, date of drop the 20th for Bramley and the 21st for Worcester.

More exactly we find:

TABLE IV.

	Bramley.				Worcester.			
	Median.	Standard Error.	Mean.	Standard Error.	Median.	Standard Error.	Mean.	Standard Error.
Flowering ..	15.50	.17	16.67	.14	16.32	.11	17.63	.09
Drop ..	20.69	.14	21.38	.11	21.70	.10	22.56	.08

We see that the precision of the experiment is more than sufficient to determine the median to the nearest day.

We also see that the mean is about a day later than the median, the distributions both of flowering and drop being perceptibly skew, rising somewhat more sharply than they fall.

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* While the median is the date by which half the flowers have come out, the mean is the average date obtained by multiplying each date by the number of flowers which came out on that date and dividing by the total number of flowers.

β_2 and β_1 which are measures of the kurtosis and skewness of the distributions, have been obtained.*

These are:

TABLE V.

		Bramley.		Worcester.	
Opening	β_2	4.98	S.E .19	3.95	S.E .12
	β_1	1.61		.96	
	$\sqrt{\beta_1}$	1.27	S.E .10	.98	S.E .06
Drop ..	β_2	4.02	S.E .19	2.70	S.E .12
	β_1	.96		.31	
	$\sqrt{\beta_1}$	.98	S.E .10	.56	S.E .06

Standard Errors calculated on basis of Normality.

All the distributions are significantly leptokurtic (i.e., with higher top and longer tails than the " normal ") with the exception of the distribution for " drop " in the Worcesters, which is platykurtic (i.e., with lower top and shorter tails than the " normal ").

All are significantly skew, as we have already seen in comparing the medians and means.

(iv) *The weather in the flowering period.*

It is of course not possible in a single season and in a single place to determine the relation of flowering to weather, nevertheless it is interesting to examine the weather of May, 1930, to see whether any interesting effects on flowering are suggested. Daily rainfall, daily maximum wind velocity and daily sunshine are shown in Fig. 15 and the maximum and minimum temperatures in Fig. 16.

The temperature, rainfall and sunshine data are given in the Appendix Table X. and the wind data, including Beaufort numbers,† at 9, 15 and 21 hours, and the time of maximum velocity in Table XI.

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* The *skewness* is of course a measure of the departure of the curve from symmetry. It is conveniently measured by the quantity $\sqrt{\beta_1}$ which is zero when the curve is symmetrical and increases as the curve becomes more and more skew. $\sqrt{\beta_1} = \frac{\mu_3}{\mu_2^{3/2}}$ where μ_3 is the mean cube of the deviation of the observed dates from the mean date and $\mu 2$ is the mean square of these deviations. Similarly β_2 is given by $\beta_2 = \frac{\mu_4}{\mu_2}{}_2$. For the " normal " frequency curve, so commonly met with, $\beta_2 = 3$ and the amount by which B_2 differs from 3 is called the " *kurtosis*." Curves with positive kurtosis or $\beta_2 > 3$ have as explained above higher tops and longer tails than the normal, for those with negative kurtosis ($\beta_2 < 3$) the reverse is the case.

† For explanation see foot of Appendix, Table XI.

There is a suggestion in Figs. 13 and 14 that the drop of Worcester flowers is more sensitive to environmental influences than the drop of Bramley's. To a lesser extent this is also suggested of the opening (Figs. 9 and 10).

It will be seen that the 14th, 15th, 16th and 17th of May were the days on which the bulk of the flowers came out. These were fine sunny or rather sunny days with maximum temperatures above 60° and minima well above 40° F. These followed a few days with rain, little sun, and lower temperatures. This suggests, as is, I suppose, a common observation, that once the buds are ready to flower, the first spell of fine warm weather will bring them out, while cool rainy weather may delay flowering for some time.

It will be seen that the 22nd-23rd May was the day on which the drop on the Worcesters reached its maximum, the maximum for the Bramley's occurring the previous day. The 22nd-23rd May covers a period from about noon on the 22nd to noon on the 23rd ; the wind velocity reached a maximum of 24 miles per hour at 7 a.m. on the 23rd and another maximum of 36 miles per hour at just after 10 a.m. on the 23rd. Both these periods come within the 22nd-23rd as defined above, thus it is easy to explain why the maximum drop on the Worcesters occurred on that day. The maximum drop on the Bramley's occurred on the 21st-22nd, on which day the maximum wind velocity was only 17 miles per hour ; thus the Bramley drop appears to be more independent of wind than that of the Worcesters, as one might expect from the less fragile nature of the flowers.

It is not easy to explain the gradual fall in numbers dropping for both varieties between the 17th and the 20th and the sudden rise in the drop on the Worcesters on the 20th-21st. Of the 17th, 18th and 19th, the 18th had the greatest maximum wind velocity, 30 miles per hour at about 5.20 p.m., otherwise the three days appear to have been about equally windy, nor do the other meteorological variates throw any obvious light on this phenomenon.

Nor is the sudden rise in the absolute and percentage numbers of Worcesters dropping on the 20th-21st easy to explain. The 20th was one of the calmest days of the month, had a relatively low maximum and high minimum temperature and only a trace of rainfall.

Thus it does not appear that this sudden increase in drop on one variety only was due to weather. It does seem on studying Figs. 13 and 14 that the drop on Worcesters is more dependent on environmental influences, but what these influences are we cannot say. If they are weather influences they must be of too subtle a nature to be revealed by an inspection of the available weather data for the month.

I am indebted to Mr. R. G. Hatton for giving me every facility in carrying out this work and to Mr. T. N. Hoblyn and numerous members of the East Malling staff for very helpful suggestions.

TABLE VI.

Worcester Pearmain. Number of Blossoms on Sample Branches—Trees W_1 and W_2.

Date.	W1.												W2.											
	N			E			S			W			N			E			S			W		
	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o
May 5th	121			138			94			82			135			93*			125			158		
,, 9th		—	—		—	—		—	—		—	—		—	—		—	—		—	—		2	—
,, 10th		—	—		2	—		—	—		—	—		6	—		—	—		2	—		4	—
,, 12th		1	—		9	—		5	—		4	—		16	—		4	—		3	—		12	—
,, 13th		6	—		13	—		9	—		4	—		19	—		7	—		9	—		14	—
,, 14th		14	—		22	—		14	—		16	—		38	—		8	—		14	—		30	—
,, 15th		23	—		44	—		40	—		19	—		72	—		14	—		31	—		53	—
,, 16th		54	—		76	—		54	2		37	—		92	—		29	—		41	—		73	—
,, 17th		76	1		94	8		53	7		45	1		105	6		37	2		70	4		89	10
,, 18th		78	9		87	21		62	11		46	8		96	29		40	11		80	9		76	37
,, 19th		73	16		86	34		50	23		51	11		83	43		37	15		68	22		71	44
,, 20th		72	24		82	39		42	31		49	13		75	53		41	18		59	31		74	48
,, 21st		56	40		63	64		29	44		36	29		55	75		35	30		47	46		64	61
,, 22nd		44	57		32	95		26	63		27	47		30	101		36	44		30	63		54	82
,, 23rd		20	81		16	111		12	77		11	63		12	120		29	51		19	75		40	104
,, 24th		18	85		7	123		11	78		10	64		11	121		32	51		15	83		45	104
,, 25th		18	95		8	127		8	81		13	64		9	125		25	68		20	90		42	111
,, 26th		14	99		6	129		8	81		12	65		8	126		25	68		16	94		32	121
,, 27th		14	100		6	129		4	86		10	67		5	129		19	75		16	96		23	131
,, 28th		8	110		3	132		8	85		8	72		—	134		13	83		6	107		7	147

TABLE VI.

Worcester Pearmain. Number of Blossoms on Sample Branches—Trees W_1 and W_2.

Date.	W₁.												W₂.											
	N			E			S			W			N			E			S			W		
	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o
May 5th	121			138			94			82			135			93*			125			158		
,, 9th		—	—		—	—		—	—		—	—		—	—		—	—		—	—		2	—
,, 10th		—	—		2	—		—	—		—	—		6	—		—	—		2	—		4	—
,, 12th		1	—		9	—		5	—		4	—		16	—		4	—		3	—		12	—
,, 13th		6	—		13	—		9	—		4	—		19	—		7	—		9	—		14	—
,, 14th		14	—		22	—		14	—		16	—		38	—		8	—		14	—		30	—
,, 15th		23	—		44	—		40	—		19	—		72	—		14	—		31	—		53	—
,, 16th		54	—		76	—		54	2		37	—		92	—		29	—		41	—		73	—
,, 17th		76	1		94	8		53	7		45	1		105	6		37	2		70	4		89	10
,, 18th		78	9		87	21		62	11		46	8		96	29		40	11		80	9		76	37
,, 19th		73	16		86	34		50	23		51	11		83	43		37	15		68	22		71	44
,, 20th		72	24		82	39		42	31		49	13		75	53		41	18		59	31		74	48
,, 21st		56	40		63	64		29	44		36	29		55	75		35	30		47	46		64	61
,, 22nd		44	57		32	95		26	63		27	47		30	101		36	44		30	63		54	82
,, 23rd		20	81		16	111		12	77		11	63		12	120		29	51		19	75		40	104
,, 24th		18	85		7	123		11	78		10	64		11	121		32	51		15	83		45	104
,, 25th		18	95		8	127		8	81		13	64		9	125		25	68		20	90		42	111
,, 26th		14	99		6	129		8	81		12	65		8	126		25	68		16	94		32	121
,, 27th		14	100		6	129		4	86		10	67		5	129		19	75		16	96		23	131
,, 28th		8	110		3	132		8	85		8	72		—	134		13	83		6	107		7	147
,, 29th		7	112		3	132		7	86		7	73		—	134		2	94		—	113		—	154
,, 30th		4	117		2	133		4	89		4	76		—	134		—	96		—	113		—	154
,, 31st		1	120		1	134		1	92		1	79		—	134		—	96		—	113		—	154
June 1st		—	121		—	135		—	93		—	80		—	134		—	96		—	113		—	154

* Clearly, a few buds were missed at the first count.

Fruit Set (June 17th).

	W₁.				W₂.			
	N	E	S	W	N	E	S	W
Good	—	—	—	3	3	—	—	—
S.F.	1	—	—	1	4	3	2	2

Number of Fruits—September 24th.

	—	—	—	—	—	—	—	—

b=initial number of buds.

f=number of flowers out.

o=number of flowers all petals of which have dropped.

S.F.=attacked by sawfly.

TABLE VIII.

Bramley's Seedling. Number of Blossoms on Sample Branches—Trees B_1 and B_2.

Date.	B1.												B2.											
	N			E			S			W			N			E			S			W		
	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o
May 5th	69			23			36			37*			47			37*			48			66		
„ 9th		—	—		—	—		—	—		—	—		—	—		—	—		—	—		1	—

	N	E	S	W	N	E	S	W
Good	6	—	8	4	1	3	5	2

Number of Fruits—September 24th.

	3 3 on ground (2 brown rot)	— (origin uncertain).	3	—	—	2 1 on ground	1 (diseased) (origin uncertain).	1

b=initial number of buds.

f=number of flowers out.

o=number of flowers all petals of which have dropped.

S.F.=attacked by sawfly.

TABLE VII.

Worcester Pearmain. Number of Blossoms on Sample Branches—Trees W_3 and W_4.

Date.	W_3.												W_4.											
	N			E			S			W			N			E			S			W		
	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o
May 5th	71			74			85			110			63			110			56			67*		
,, 9th		—	—		—	—		—	—		—	—		—	—		—	—		—	—		1	—
,, 10th		—	—		—	—		—	—		—	—		—	—		—	—		—	—		1	—
,, 12th		—	—		—	—		—	—		—	—		2	—		1	—		1	—		2	—
,, 13th		—	—		1	—		—	—		1	—		2	—		3	—		1	—		2	—
,, 14th		1	—		4	—		5	—		8	—		6	—		15	—		3	—		4	—
,, 15th		10	—		10	—		12	—		19	—		10	—		36	—		4	—		7	—
,, 16th		18	—		20	—		22	—		41	—		27	—		51	—		13	—		14	—
,, 17th		37	—		35	—		46	—		76	—		39	1		73	—		16	—		20	—
,, 18th		46	1		54	2		56	5		81	13		42	2		70	11		26	3		23	5
,, 19th		41	6		50	8		56	10		90	13		41	9		70	17		25	6		23	7
,, 20th		47	13		63	9		58	11		86	17		36	15		68	30		25	10		24	8
,, 21st		41	20		44	28		50	19		75	28		33	20		43	57		15	20		21	11
,, 22nd		33	29		36	36		37	35		41	62		27	27		28	72		20	22		17	16
,, 23rd		14	48		26	46		18	60		22	81		13	45		12	91		13	35		10	26
,, 24th		16	48		20	52		17	63		12	94		9	49		11	93		15	35		12	34
,, 25th		9	55		13	59		13	67		6	100		4	54		4	100		12	38		7	39
,, 26th		7	57		6	66		11	69		3	103		4	54		1	103		13	39		12	42
,, 27th		5	60		7	66		9	72		—	106		—	58		—	104		7	46		25	42
,, 28th		4	63		2	71		4	79		—	106		—	58		—	104		1	53		22	50
,, 29th		2	66		—	73		1	82		—	106		—	58		—	104		—	54		13	61
,, 30th		—	68		—	73		—	83		—	106		—	58		—	104		—	54		—	74

* Clearly, a few buds were missed at the first count.

Fruit Set (June 17th).

	W_3.				W_4.			
	N	E	S	W	N	E	S	W
Good	—	—	1	2	1	1	—	—
S.F.	—	1	—	4	1	2	—	—

Number of Fruits—September 24th.

	—	—	—	—	— 1 on ground (diseased)	— (not certain from which branch)	—	—

b=initial number of buds.

f=number of flowers out.

o=number of flowers all petals of which have dropped.

S.F.=attacked by sawfly.

TABLE VIII.

Bramley's Seedling. Number of Blossoms on Sample Branches—Trees B_1 and B_2.

Date.	B1.												B2.											
	N			E			S			W			N			E			S			W		
	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o
May 5th	69			23			36			37*			47			37*			48			66		
,, 9th		—	—		—	—		—	—		—	—		—	—		—	—		—	—		1	—
,, 10th		—	—		—	—		—	—		1	—		—	—		—	—		—	—		3	—
,, 12th		—	—		—	—		3	—		2	—		1	—		—	—		—	—		8	—
,, 13th		2	—		1	—		5	—		4	—		4	—		1	—		—	—		14	—
,, 14th		5	—		1	—		7	—		9	—		7	—		1	—		3	—		19	—
,, 15th		12	—		4	—		13	—		16	—		18	—		8	—		14	—		24	—
,, 16th		16	—		5	—		19	—		20	—		28	—		10	—		29	—		31	—
,, 17th		19	—		10	—		23	—		23	—		31	—		19	—		38	—		38	3
,, 18th		22	4		11	1		18	6		18	7		34	4		23	—		39	—		29	13
,, 19th		19	7		11	3		16	10		13	13		29	9		20	5		36	3		30	13
,, 20th		19	16		7	9		10	17		14	16		26	13		22	9		34	6		24	19
,, 21st		10	25		3	13		9	18		9	21		20	21		20	11		33	13		23	23
,, 22nd		8	28		2	14		6	21		6	24		11	32		13	18		22	24		16	30
,, 23rd		1	35		—	16		3	24		3	29		5	38		7	24		4	43		6	40
,, 24th		3	35		—	16		1	26		5	29		3	40		3	28		3	44		5	42
,, 25th		2	36		—	16		2	26		8	29		1	42		1	40		1	46		4	43
,, 26th		2	36		—	16		2	26		8	29		1	42		1	40		1	46		4	43
,, 27th		1	37		—	16		2	27		8	29		—	43		—	41		—	47		4	43
,, 28th		2	37		—	16		2	28		5	33		—	43		—	41		—	47		—	47
,, 29th		1	38		1	16		2	28		—	38		—	43		—	41		—	47		—	47
,, 30th		2	38		1	16		1	29		—	38		—	43		—	41		—	47		—	47
,, 31st		—	40		—	17		—	30		—	38		—	43		—	41		—	47		—	47

* Clearly a few buds were missed at the first count.

Fruit Set (June 17th).

	B1.				B2.			
	N	E	S	W	N	E	S	W
Good	6	—	8	4	1	3	5	2

Number of Fruits—September 24th.

	3 3 on ground (2 brown rot)	— (origin uncertain).	3	—	—	2 1 on ground	1 (diseased) (origin uncertain).	1

b=initial number of buds.

f=number of flowers out.

o=number of flowers all petals of which have dropped.

S.F.=attacked by sawfly.

TABLE IX.

Bramley's Seedling. Number of Blossoms on Sample Branches—Trees B_3 and B_4.

Date.	B3.												B4.											
	N			E			S			W			N			E			S			W		
	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o
May 5th	52			44			54*			80			33			33			56			18		
,, 9th		—	—		—	—		—	—		—	—		—	—		—	—		—	—		—	—
,, 10th		—	—		—	—		—	—		2	—		—	—		—	—		1	—		—	—
,, 12th		2	—		2	—		2	—		7	—		4	—		1	—		5	—		—	—
,, 13th		5	—		2	—		8	—		7	—		6	—		2	—		7	—		—	—
,, 14th		9	—		6	—		9	—		22	—		10	—		9	—		17	—		2	—
,, 15th		25	—		16	—		22	—		37	—		21	—		13	—		24	—		5	—
,, 16th		33	—		24	—		32	—		39	—		26	—		25	—		28	—		8	—
,, 17th		37	—		28	—		35	—		54	—		25	1		31	—		41	2		10	—
,, 18th		34	3		25	4		41	8		35	19		18	8		25	8		42	13		11	1
,, 19th		26	12		25	6		31	18		26	28		10	16		18	15		36	19		7	7
,, 20th		27	18		21	12		26	25		17	37†		9	17		11	22†		32	23		9	7†
,, 21st		20	25		12	21		22	30		9	46†		8	18		9	24		25	30		8	8
,, 22nd		9	36		3	30		6	48		9	46†		3	23		5	28		15	40		4	12
,, 23rd		3	42		3	31		2	52		1	56		3	24		—	33		11	44		—	16
,, 24th		2	44		1	33		1	53		1	56		2	25		—	33		6	50		—	16
,, 25th		4	45		—	34		2	53		—	57		1	26		—	33		1	55		—	16
,, 26th		5	47		—	34		3	55		—	57		2	27		—	33		—	56		—	16
,, 27th		5	47		—	38		3	55		—	57		2	27		—	33		—	56		—	16
,, 28th		3	49		—	38		1	57		—	57		—	29		—	33		—	56		—	16
,, 29th		—	52		—	38		2	58		—	57												
,, 30th		—	52		—	38		1	59		—	57												
,, 31st		—	52		—	38		—	60		—	57												

* Clearly, a few buds were missed at first count. † Estimated.

Fruit Set (June 17th).

	B3.				B4.			
	N	E	S	W	N	E	S	W
Good	3	1	4	3	1	4	3	—
S.F.	—	2	—	—	—	—	2	—

Number of Fruits—September 24th.

	2	*Nil.*	1+2 on ground also two brown-rotted on ground	*Nil.* origin uncertain	1 1 undeveloped on ground	3 origin uncertain	1	—

b=initial number of buds.

f=number of flowers out.

o=number of flowers all petals of which have dropped.

S.F.=attacked by sawfly.

TABLE X.

Weather Data—May, 1930. *(East Malling.)*

Date.	Rainfall inches.	Sunshine hours.	Temperature (F.).	
			Max.	Min.
May 1st	—	10.1	57	39
,, 2nd	trace	3.8	59	41
,, 3rd	.01	1.7	58	41
,, 4th	—	2.4	63	42
,, 5th	.26	6.6	64	33
,, 6th	.445	4.2	59	45
,, 7th	.005	2.7	51	43
,, 8th	—	9.4	52	38
,, 9th	.32	6.5	55	37
,, 10th	.14	5.6	55	41
,, 11th	.06	0.8	55	43
,, 12th	.09	5.6	58	45
,, 13th	.07	0.0	60	47
,, 14th	—	12.2	65	48
,, 15th	—	4.0	61	41
,, 16th	—	8.9	66	44
,, 17th	—	2.9	64	47
,, 18th	.215	10.3	61	50
,, 19th	—	6.4	59	39
,, 20th	trace	0.0	58	50
,, 21st	trace	6.6	59	47
,, 22nd	.30	1.6	57	43
,, 23rd	—	10.5	63	45
,, 24th	.19	2.9	62	47
,, 25th	.18	0.0	55	49
,, 26th	.10	1.3	62	50
,, 27th	—	10.3	68	45
,, 28th	—	13.4	71	42
,, 29th	—	11.5	71	44
,, 30th	.83	1.4	67	49
,, 31st	.23	3.9	64	54

TABLE XI.

Records of Wind—May, 1930. (East Malling.)

Date.		Beaufort Numbers.* 9 hrs.	15 hrs.	21 hrs.	Maximum Wind Velocity (m.p.h.) Velocity.	Time of Occurrence.†
May	1st	4	4	1	30	15.6
,,	2nd	1	1	calm	10	15.6
,,	3rd	1	calm	calm	9	12.7
,,	4th	calm	1	calm	14	18.5
,,	5th	1	2	calm	20	18.2
,,	6th	2	2	calm	21	1.0
,,	7th	3	3	calm	24	4.6
,,	8th	4	3	1	20	11.8
,,	9th	4	4	3	30	16.5
,,	10th	1	4	3	23	8.2
,,	11th	4	4	3	28	15.6
,,	12th	4	4	1	22	12.9
,,	13th	3	3	3	19	16.6 8.8
,,	14th	4	3	calm	21	11.8 12.3
,,	15th	3	4	2	20	15.0
,,	16th	3	2	1	17	9.7 16.9
,,	17th	3	4	1	26	0.8
,,	18th	4	3	2	30	17.3
,,	19th	4	4	2	20	12.8
,,	20th	1	2	calm	15	12.3
,,	21st	3	3	1	17	8.5
,,	22nd	3	4	3	24	7.0
,,	23rd	5	5	3	36	10.9
,,	24th	2	1	calm	16	2.3
,,	25th	3	1	calm	11	9–10
,,	26th	calm	calm	calm	5	18.6
,,	27th	calm	1	1	12	13.9
,,	28th	4	3	calm	20	10.7
,,	29th	3	1	calm	13	12.7
,,	30th	2	2	3	29	18.5
,,	31st	3	3	1	18	11.3

* Beaufort No. 1 corresponds to 1— 3 miles per hour at 33 ft. in the open.
2 ,, ,, 4— 7 ,, ,, ,, ,, ,,
3 ,, ,, 8—12 ,, ,, ,, ,, ,,
4 ,, ,, 13—18 ,, ,, ,, ,, ,,
5 ,, ,, 19—24 ,, ,, ,, ,, ,,

† The day is counted from 9 a.m. to 9 a.m. for this purpose, thus 17 hrs. against the 21st means 5 p.m. on the 21st, 5 hrs. against the 6th means 5 a.m. on the 7th. 10 hrs. against the 2nd means 10 a.m. on the 2nd, etc.

Time given to the nearest tenth of an hour, except on the 25th when there was a level trace on the chart between 9 and 10.

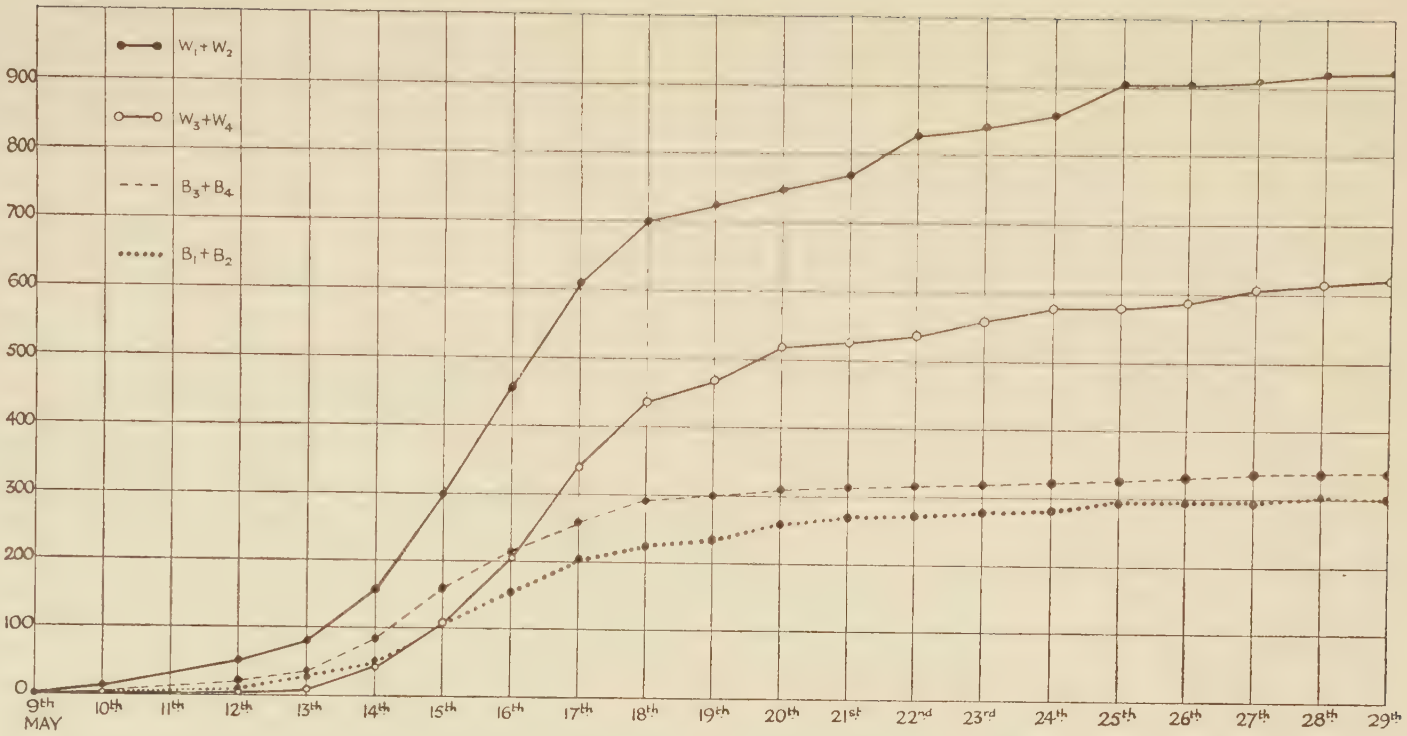


FIG. 1.

No. of Flowers which have opened by stated dates (Trees on East [1 and 2] and West [3 and 4] compared).

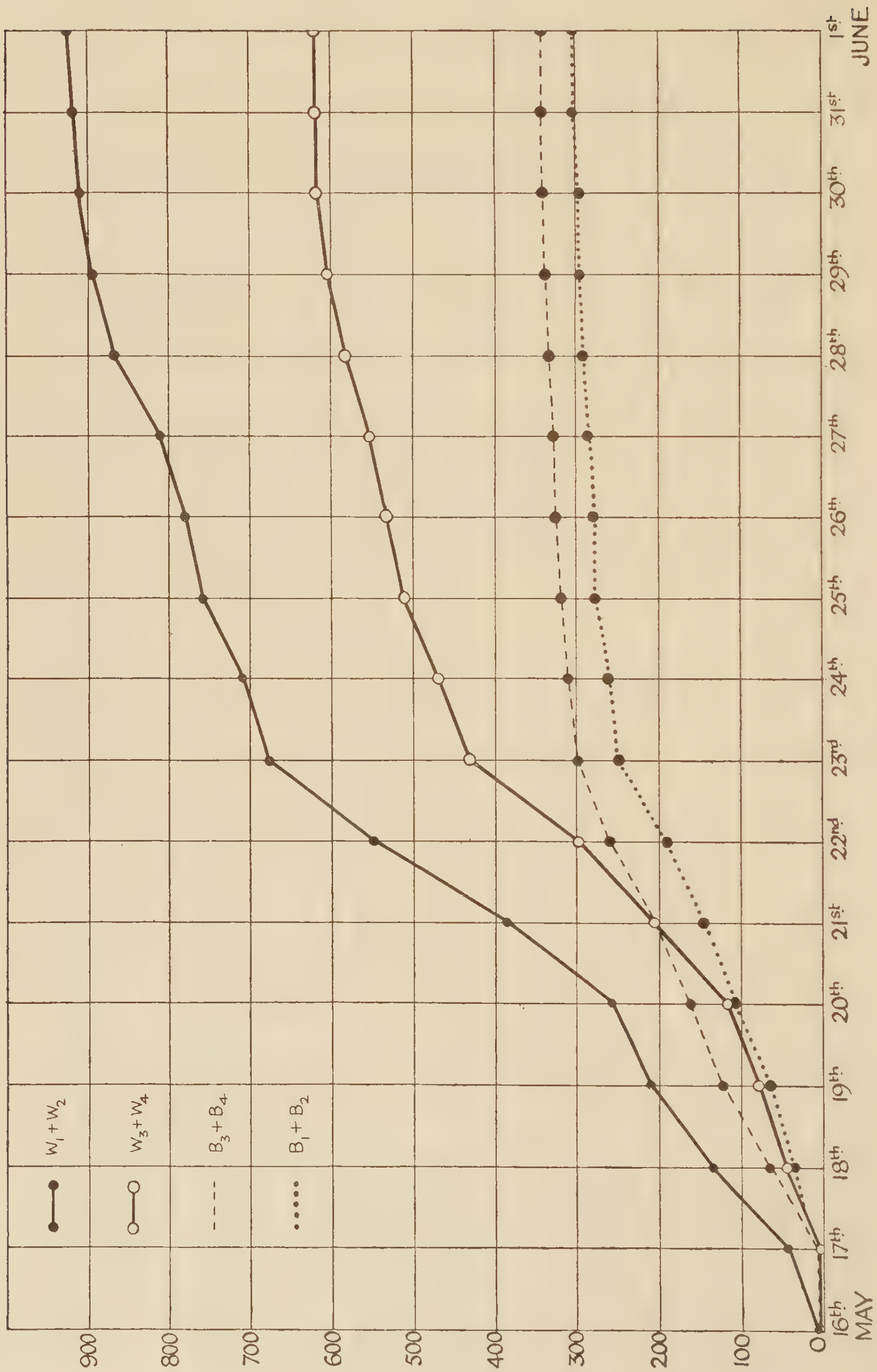


Fig. 2.

No. of Flowers which have dropped (all petals) by stated dates (Trees on East [1 and 2] and West [3 and 4] compared).

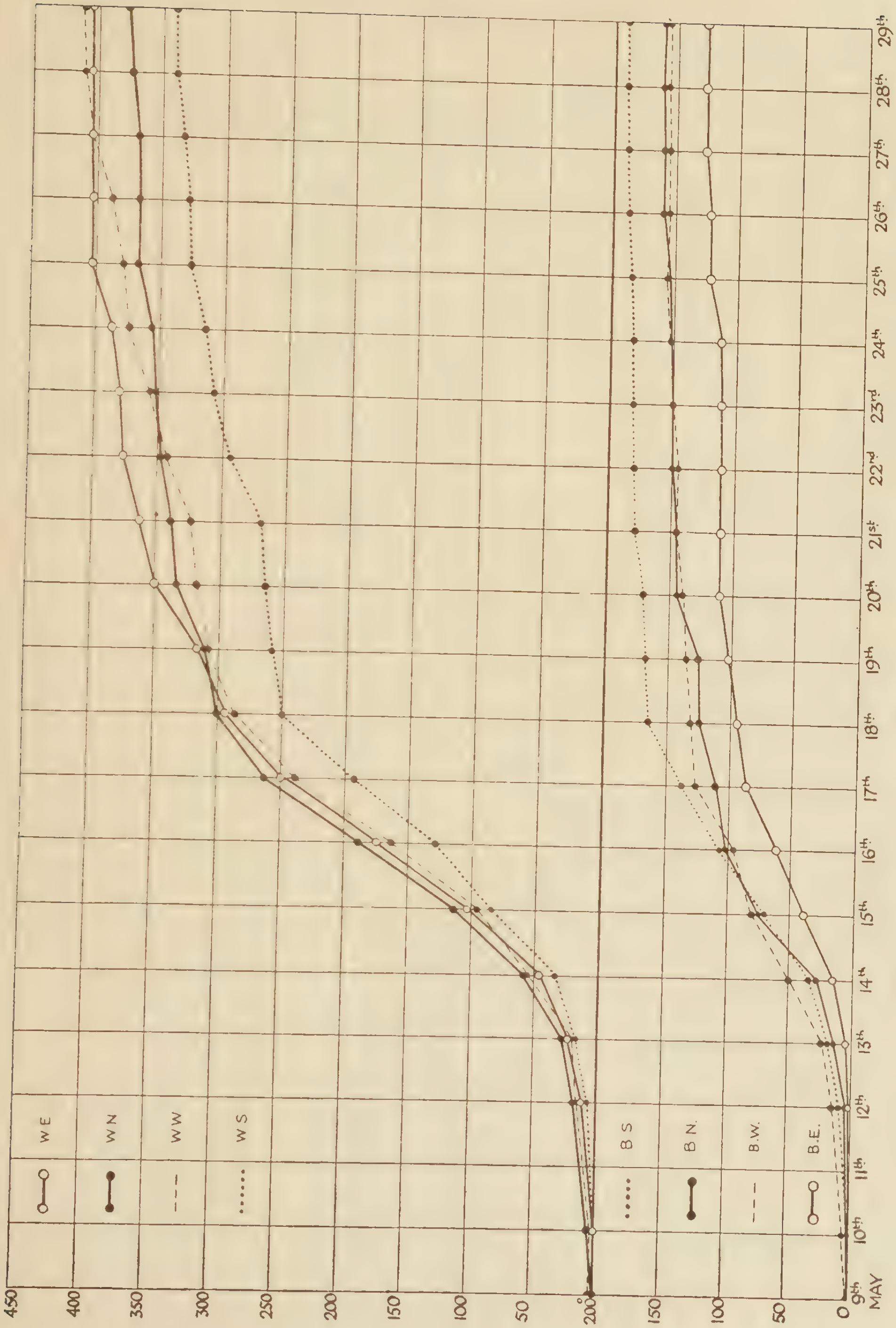


FIG. 3.

No. of Flowers which have opened by stated dates (samples in different geographical positions compared).

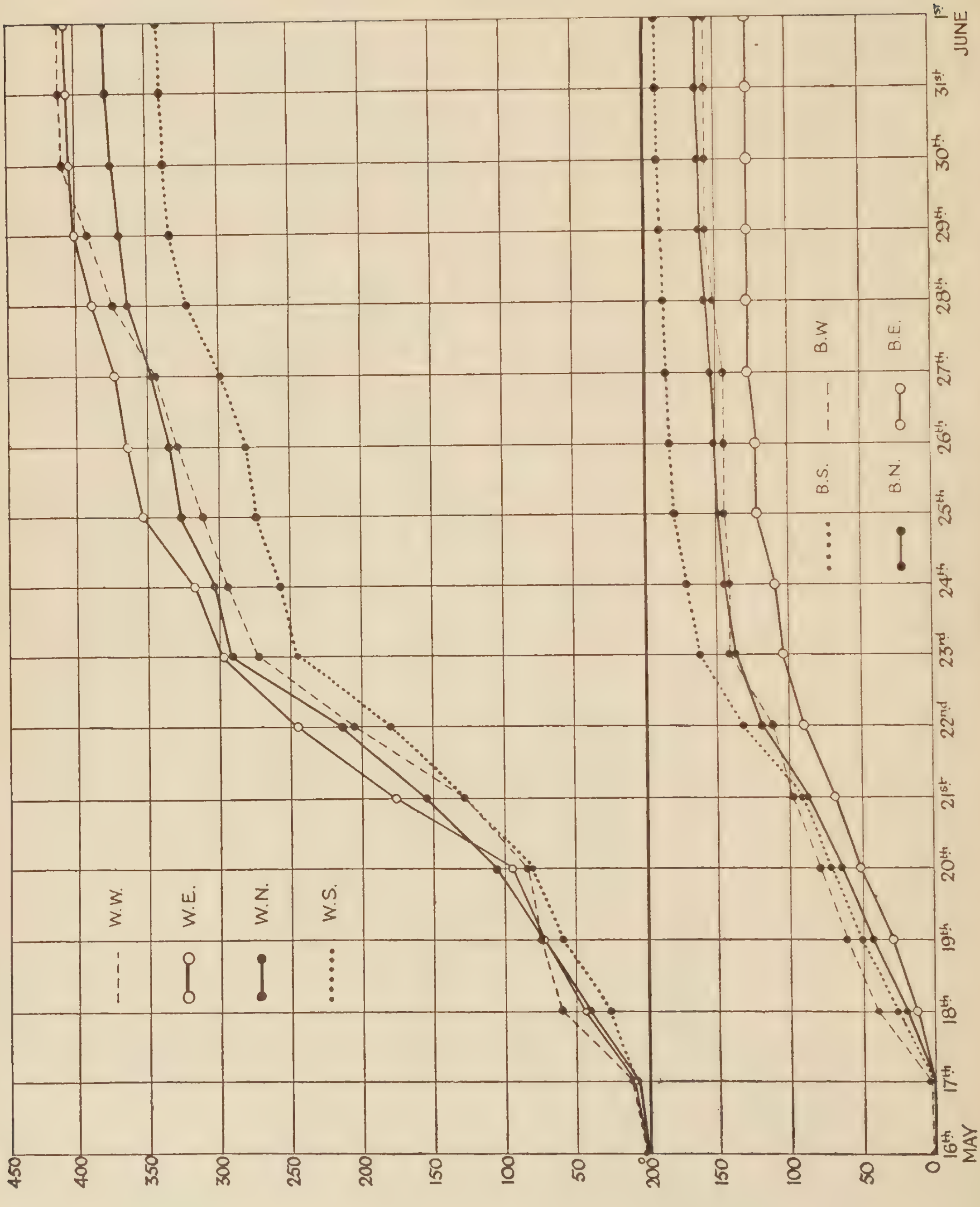


Fig. 4.

No. of Flowers which have dropped (all petals) by stated dates (samples in different geographical positions compared).

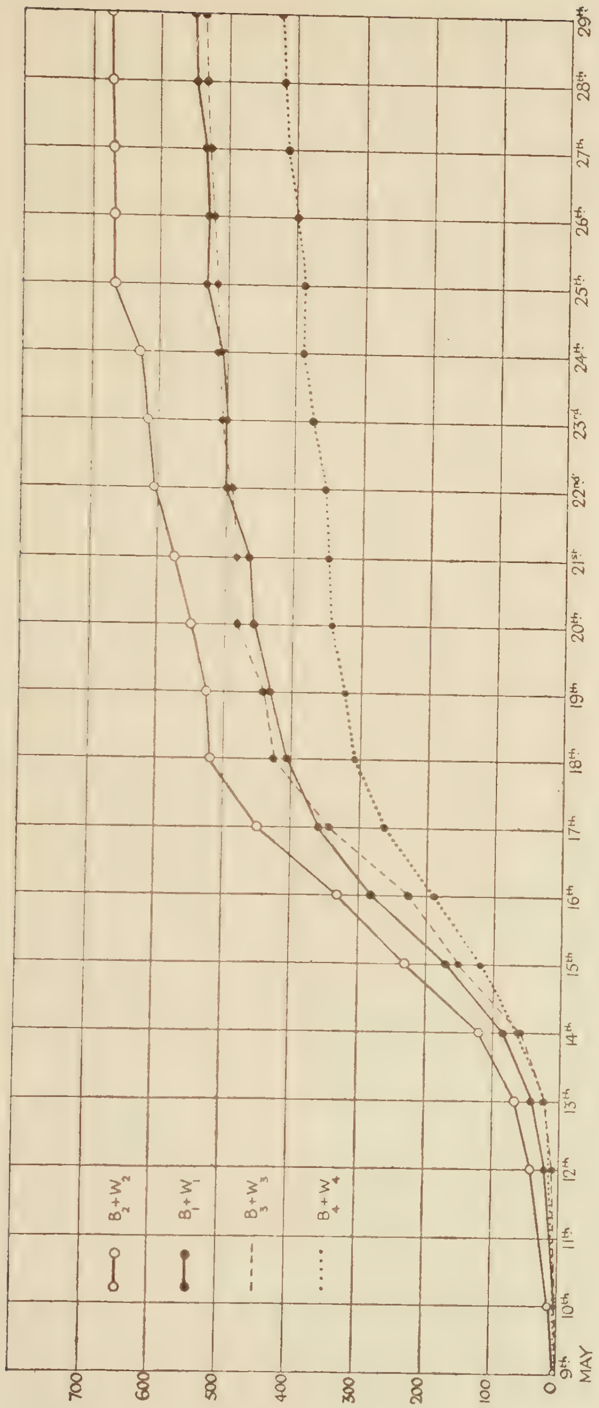


FIG. 5.

No. of Flowers which have opened by stated dates (positions in plot compared).

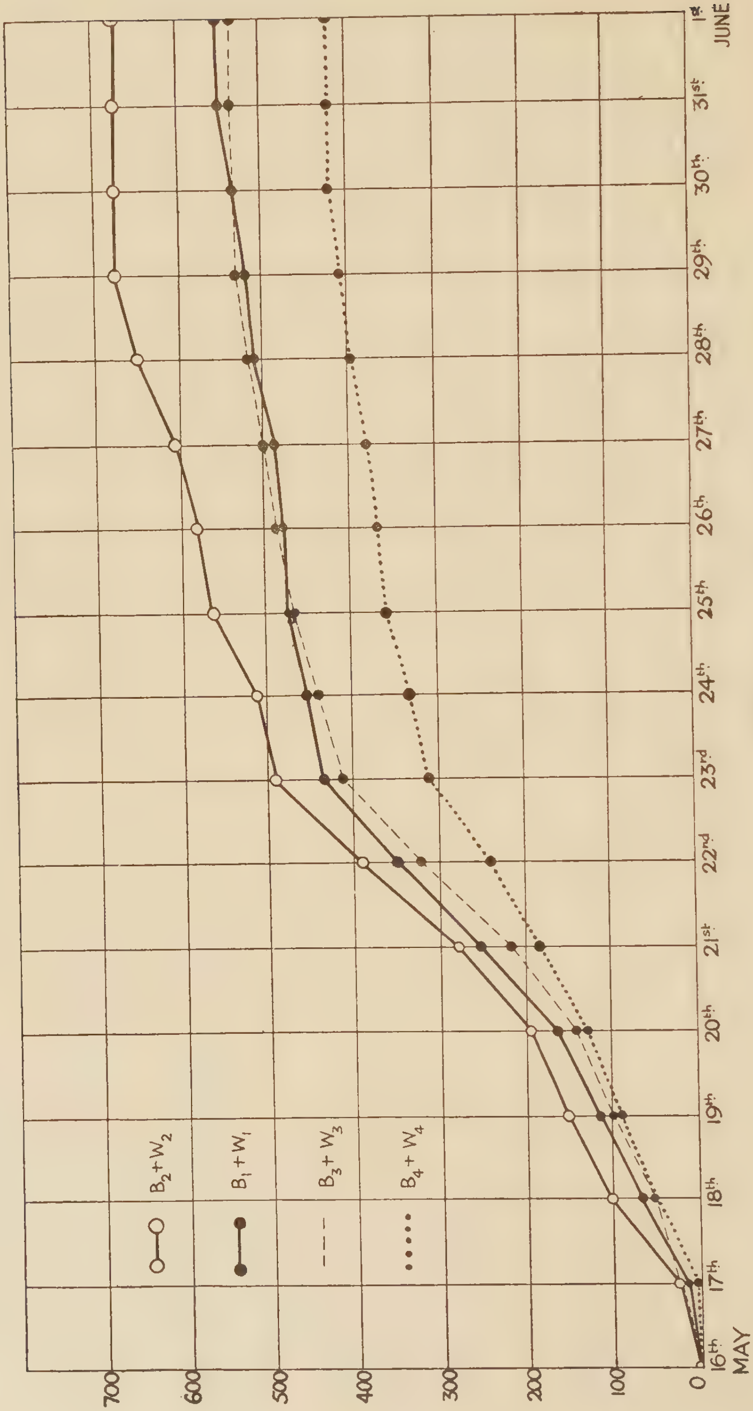


Fig. 6.

No. of Flowers which have dropped (all petals) by stated dates (positions in plot compared).

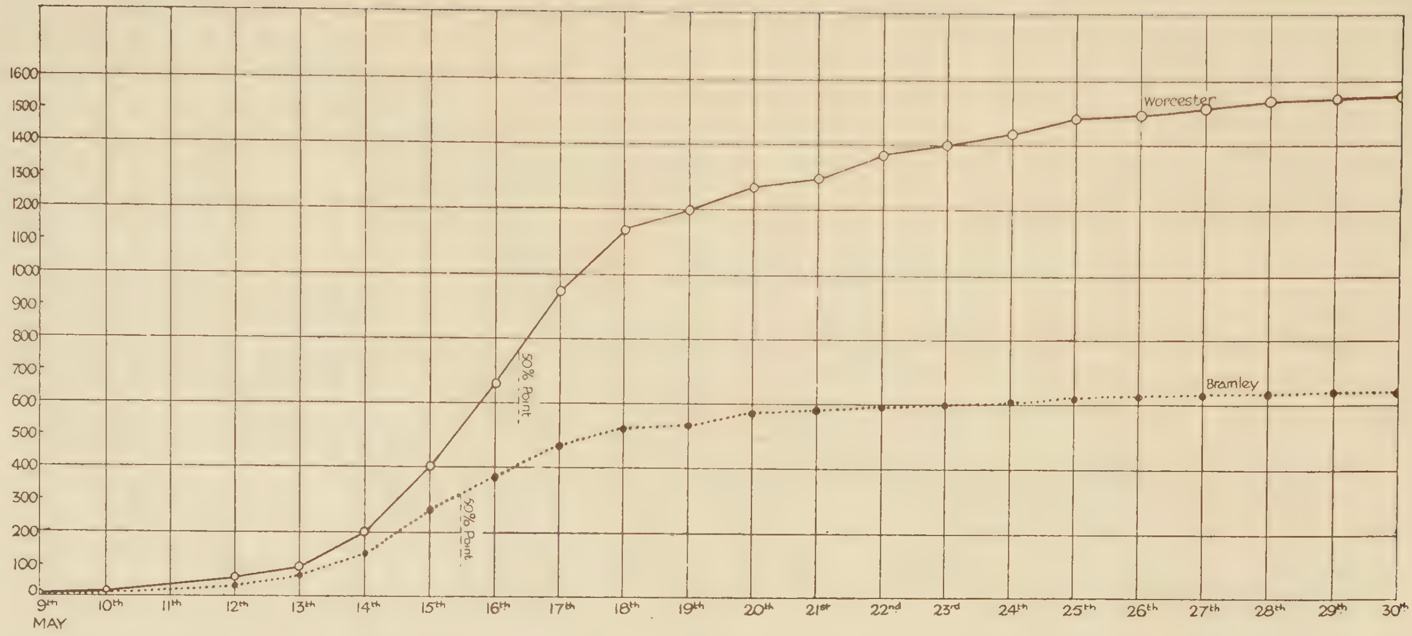


FIG. 7.
No. of Flowers which have opened by stated dates.

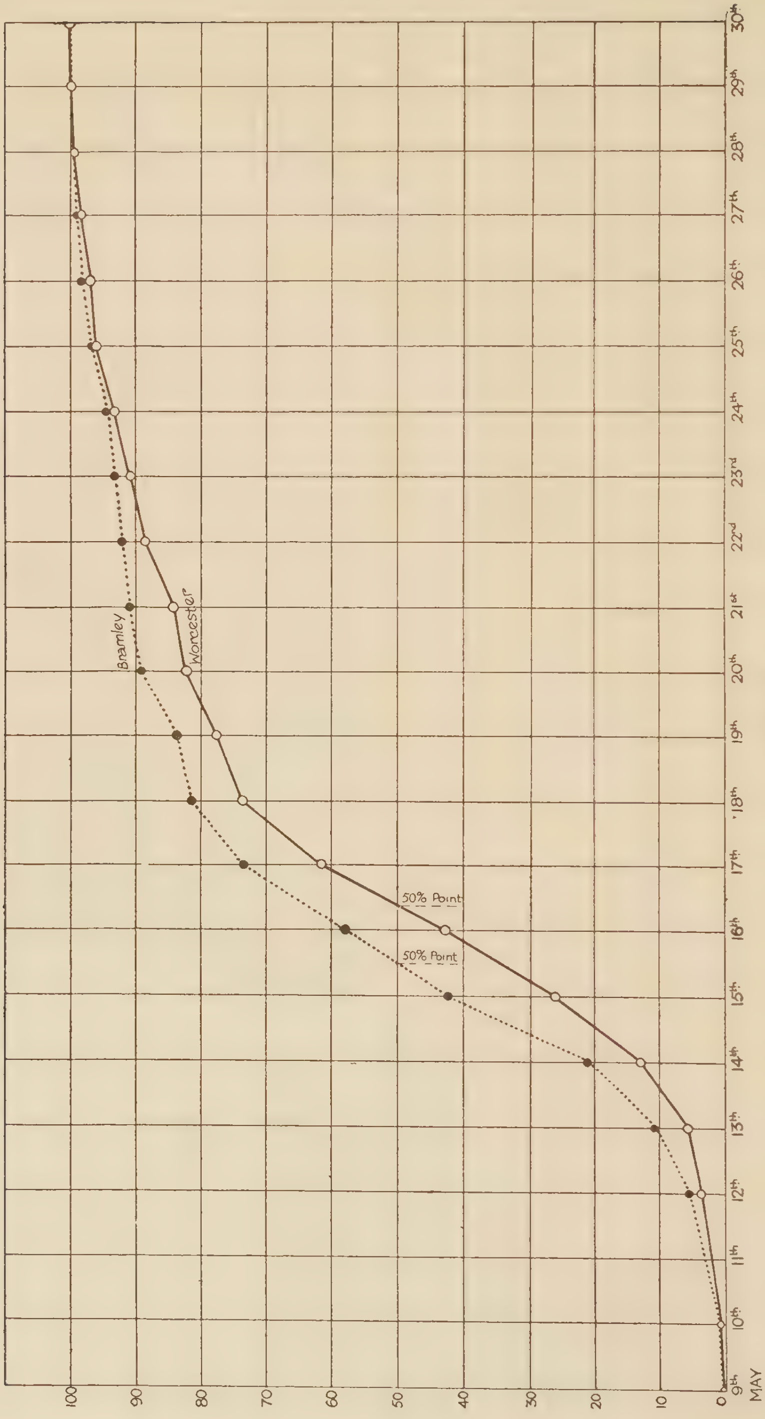


Fig. 8.

Percentage of Flowers which have opened by stated dates.

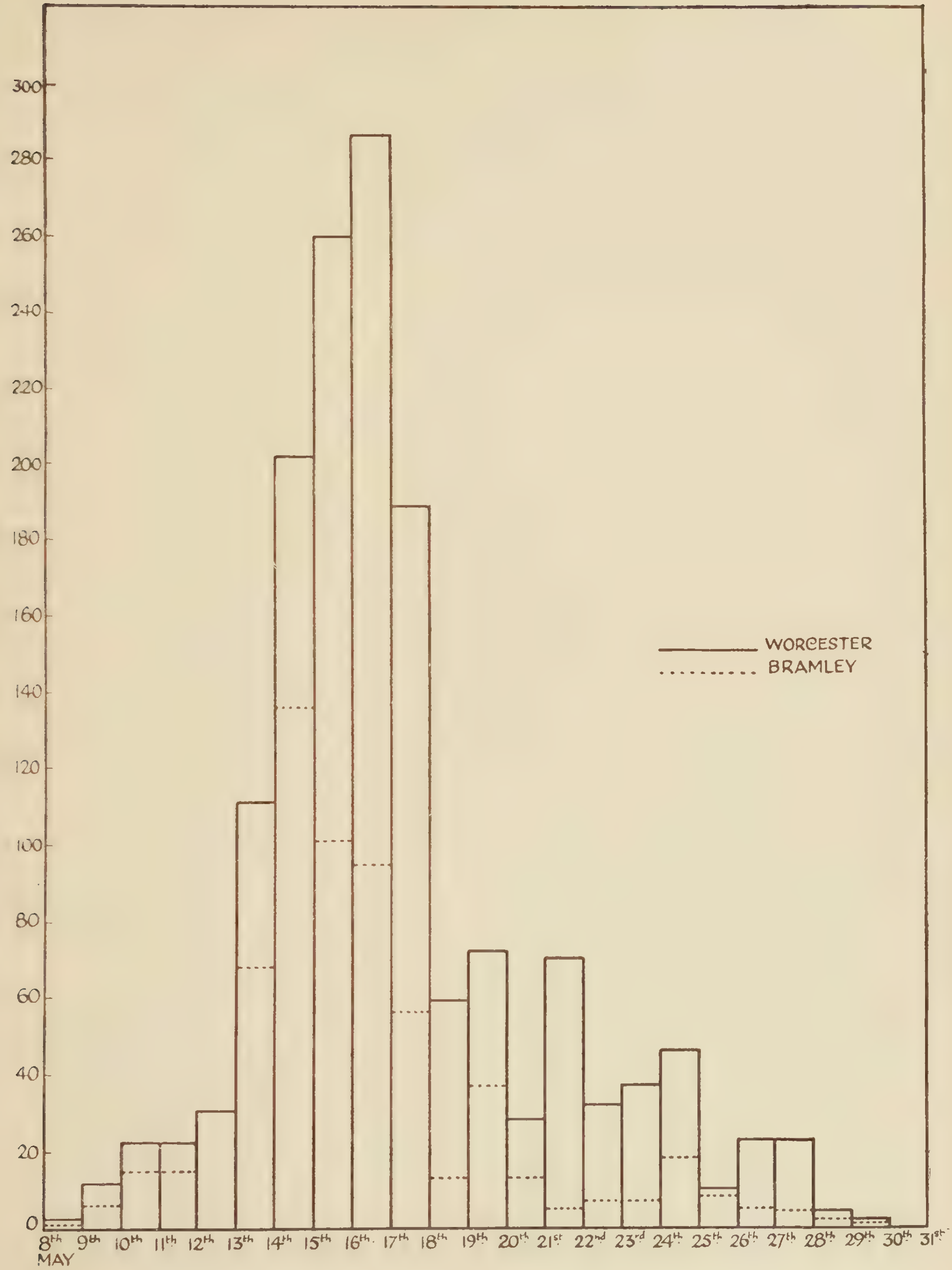


FIG. 9.
Frequency Distribution of Time of Flowering (actual frequencies).
No. of Flowers which opened each day.

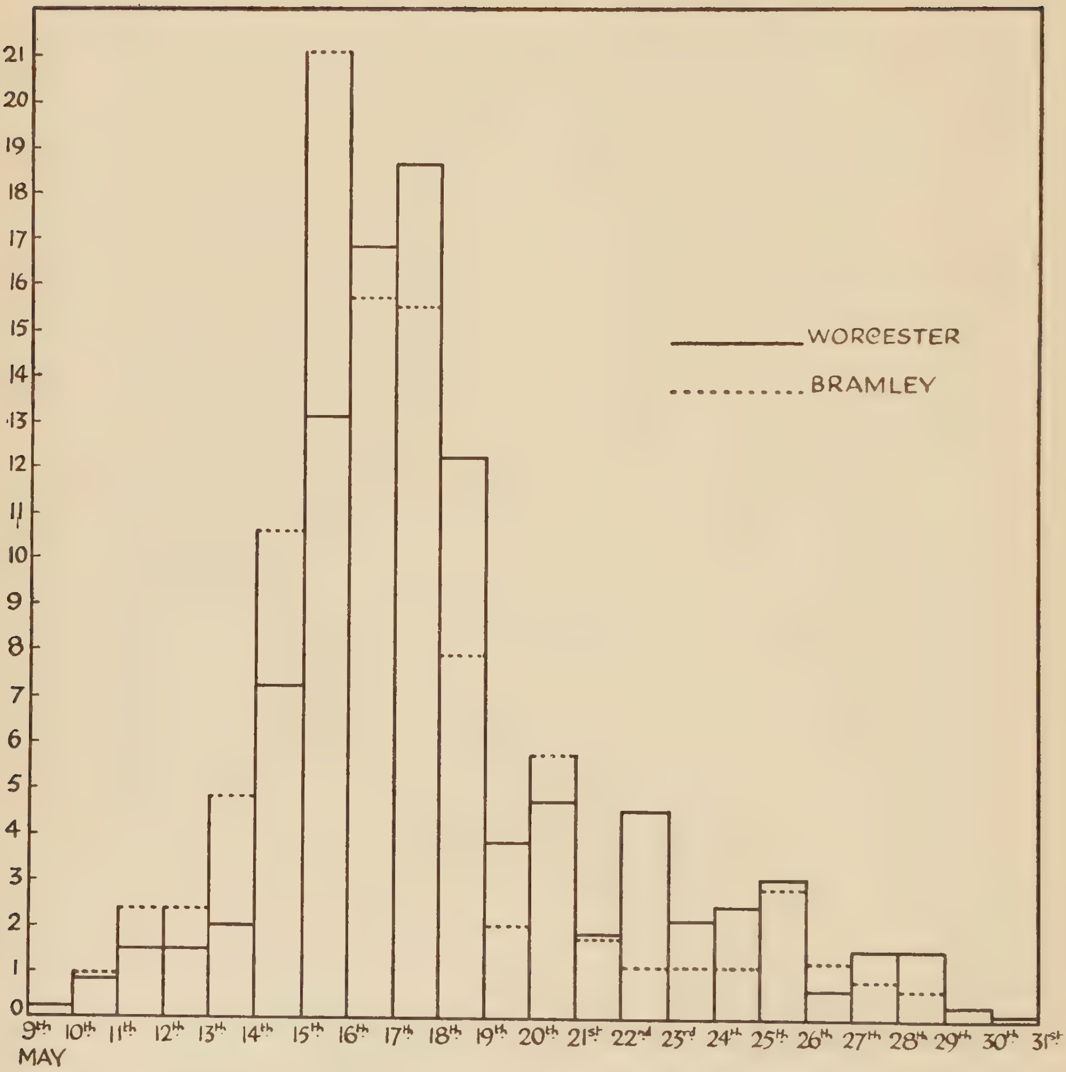


FIG. 10

Frequency Distribution of Time of Flowering (percentage frequencies).
Percentage of Flowers which opened each day.

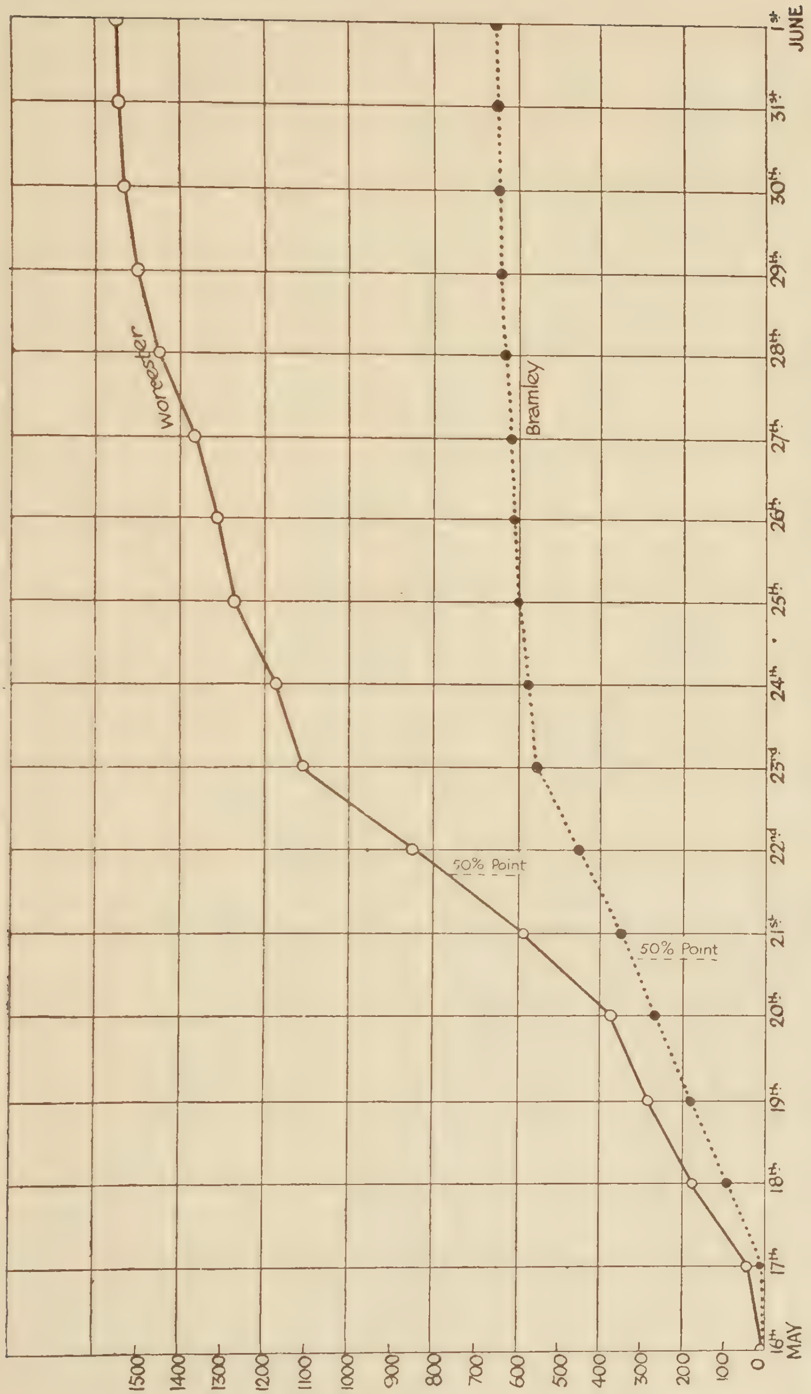


FIG. 11.

No. of Flowers which have dropped (all petals) by stated dates.

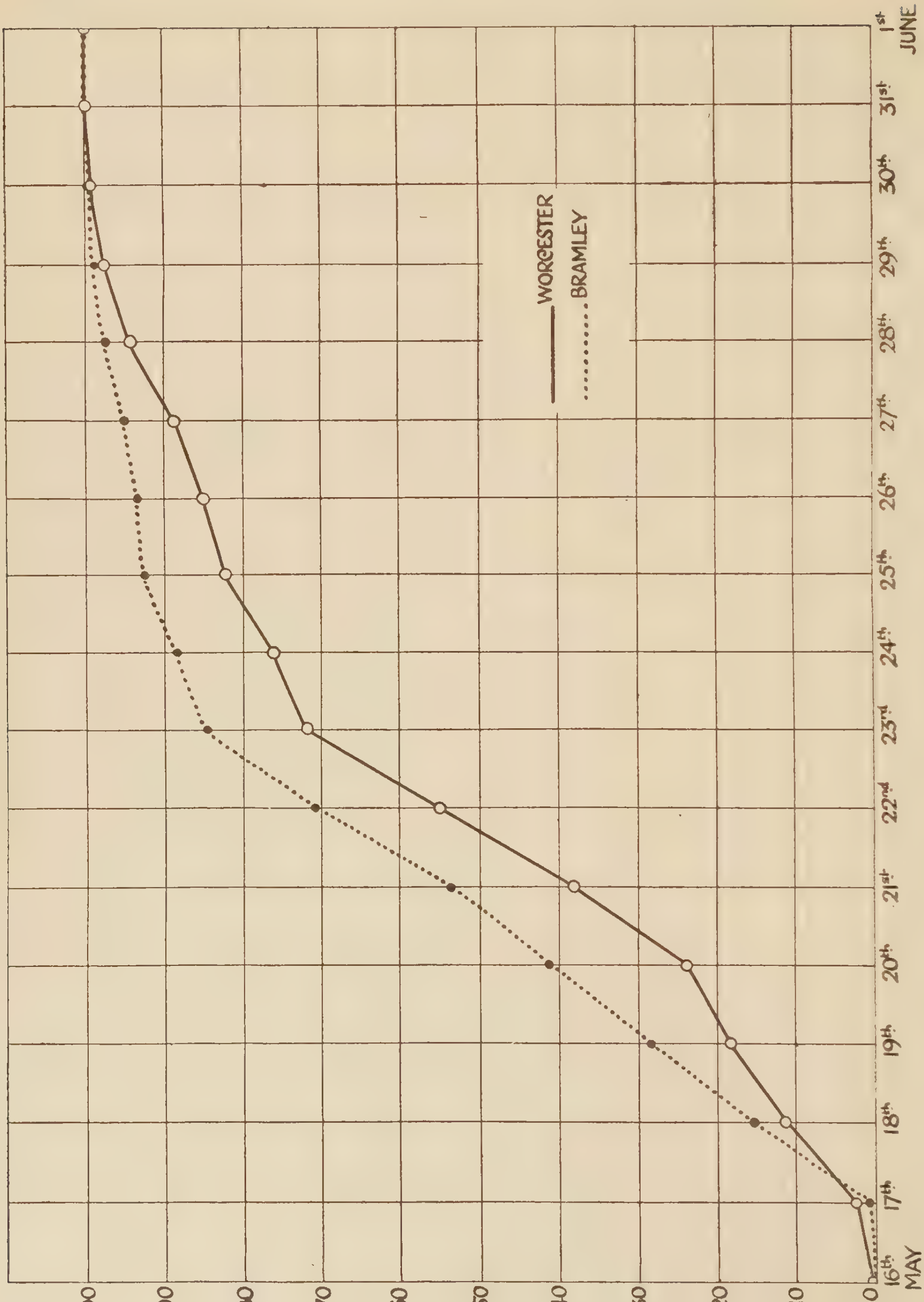


Fig. 12.

Percentage of Flowers which have dropped (all petals) by stated dates.

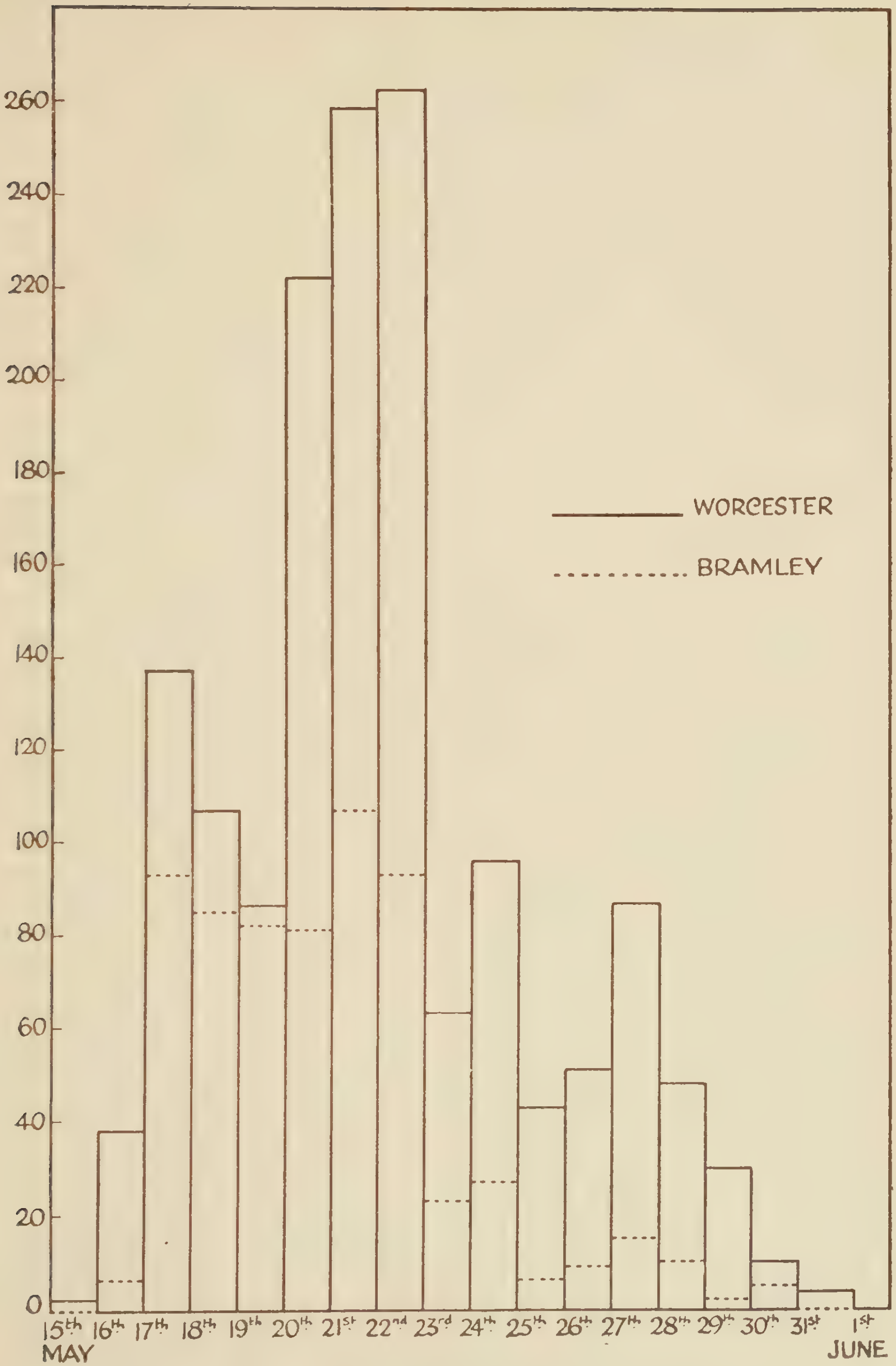


FIG. 13.

Frequency Distribution of Time of Drop (actual frequencies).
No. of Flowers dropping (all petals) each day.

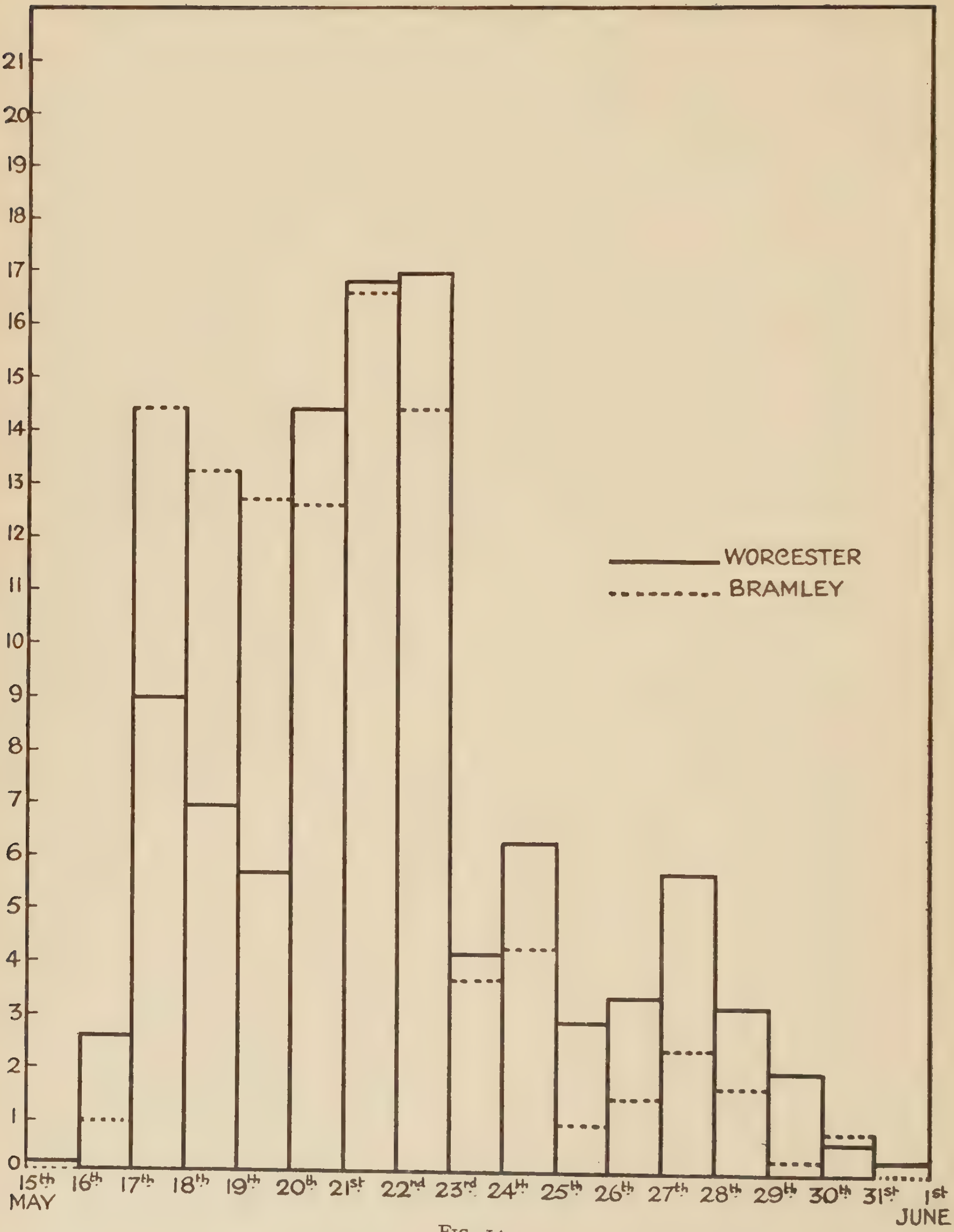


FIG. 14.

Frequency Distribution of Drop (percentage frequencies).
Percentage of Flowers dropping (all petals) each day.

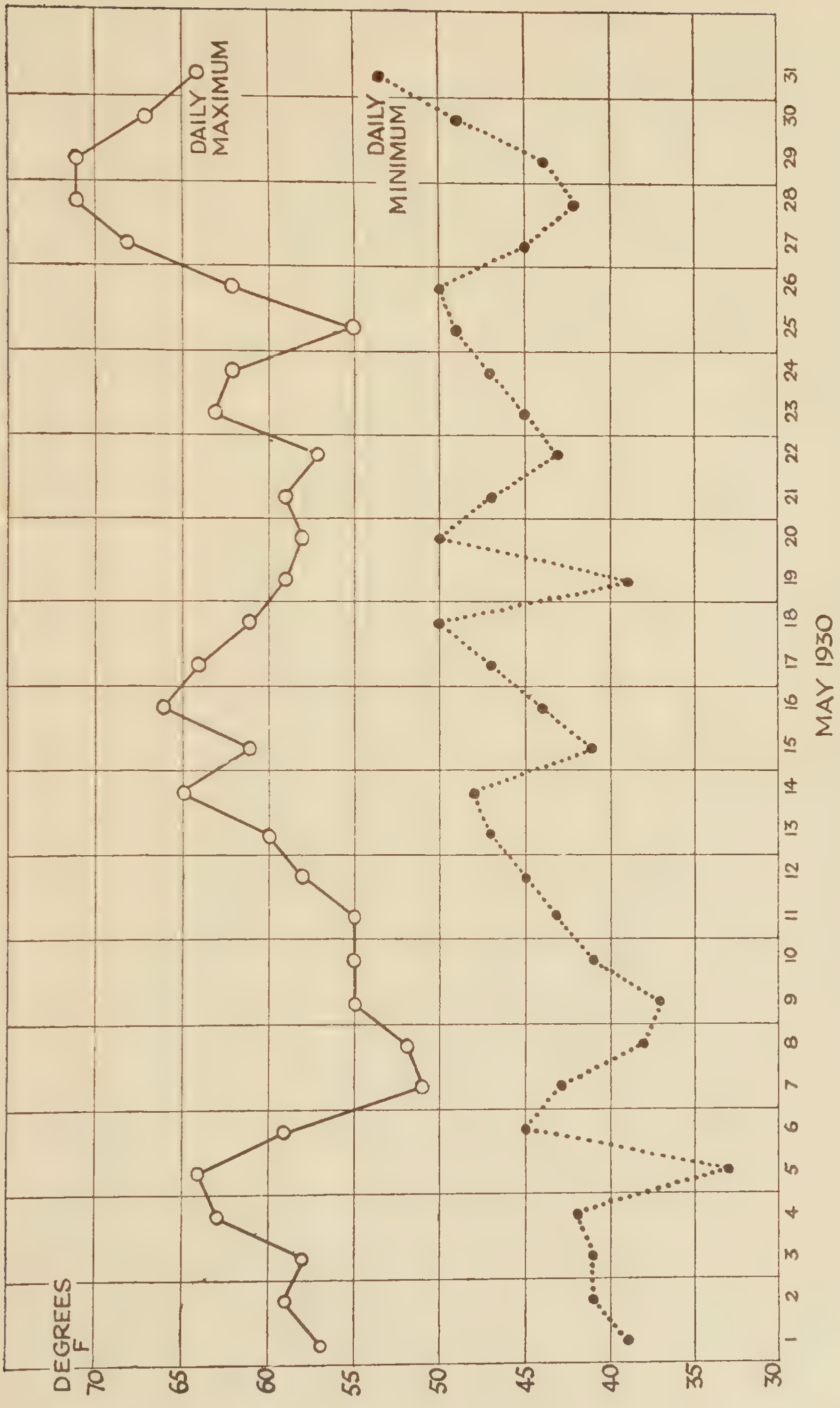


Fig. 15.

Daily Maximum and Minimum Temperatures—May 1930.

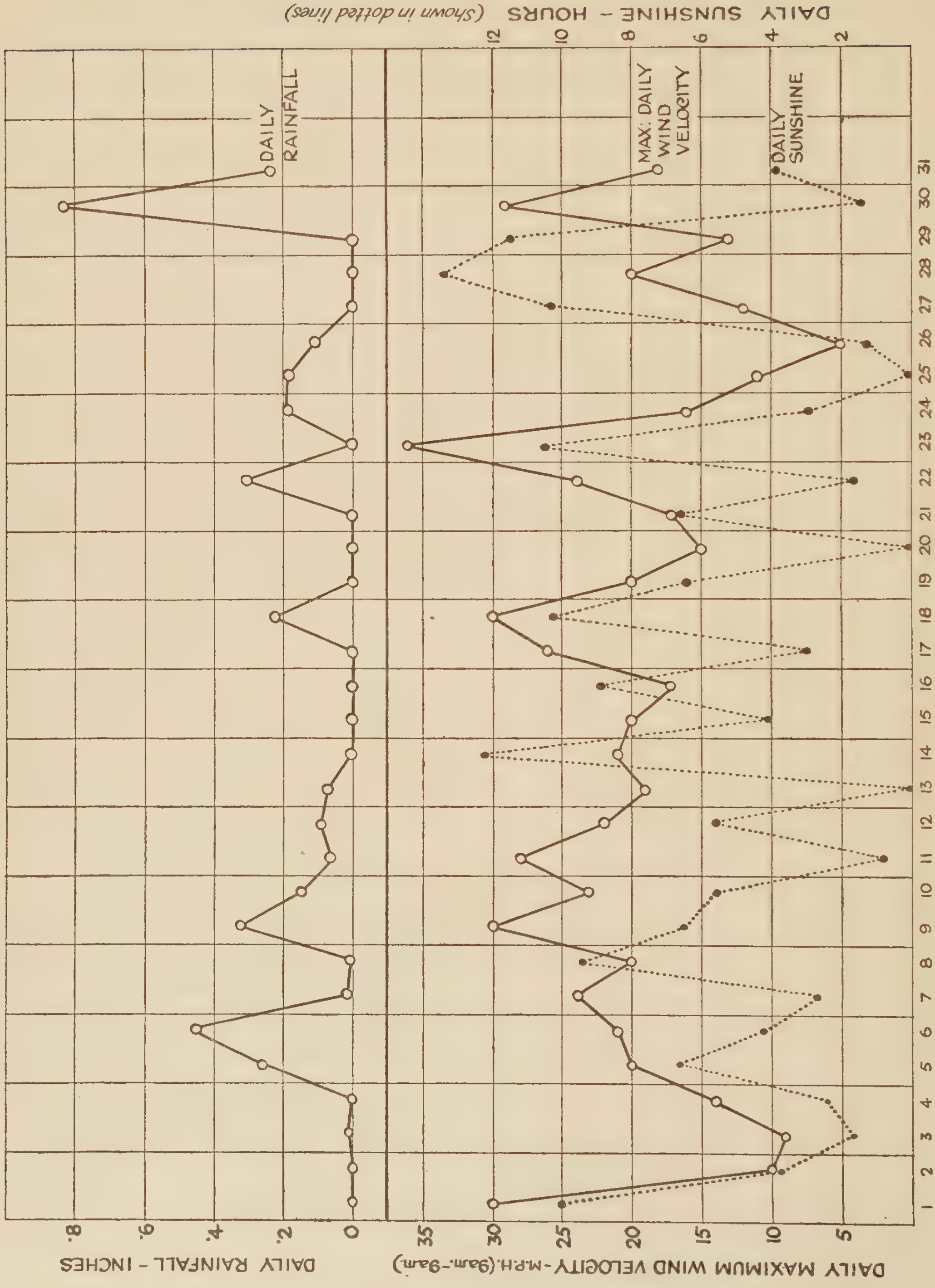


Fig. 16.
Rainfall, Wind and Sunshine—May, 1930.

(*Received* 19/12/30.)

OBSERVATIONS ON THE VARIETAL RESISTANCE OF THE APPLE TO SCAB (*Venturia inaequalis,* Aderh.) WITH SPECIAL REFERENCE TO ITS PHYSIOLOGICAL ASPECTS.

By K. H. JOHNSTONE, B.A., Ph.D.

Part II.

A study of the toxity of liquids expressed from the apple plant in relation to the fungus.

INTRODUCTORY.

Some account has been given in Part I. of this paper of the requirements of the fungus *Venturia inaequalis* for spore germination and of ways in which differences in the surface of the host plant may affect the parasite. Observations on variations in the vitality of the parasite suggested the existence of specially virulent strains and some biological specialisation of strains. Penetration of the cuticle by an infection hypha normally follows germination, but appears to be prevented by the nature of the cuticle in certain varieties.

Infection of the host occurs when the fungus reaches a favourable environment beneath the cuticle, and is able to form a " subcuticular pad." The chemical nature of the fluids of the epidermal tissues appears to be the primary factor determining the suitability of the environment for the establishment of the fungus. Occluding barriers of an anatomical nature are probably of subsidiary importance.

In this connection Wiltshire (1922) observed that *Venturia inaequalis*, in contrast to *Nectria galligena*, Bres, is able to penetrate a cork barrier, so that varietal differences in phellogen development should be of no great importance. The present writer would suggest, however, that rapid cork formation causing the epidermal cells and cuticle to disappear before the fungus is well established, is a factor in preventing the formation of large lesions. Such cork formation occurs in the fruit of Medaille d'Or and in varieties of the russet type of apple. Scab may be seen on Medaille d'Or fruits, but a badly scabbed and cracked apple is rarely observed. The resistance of russet apples is well known. Once a corky surface has replaced the cuticle, considerable resistance to penetration may be offered ; the infection hypha may have less power than a hyphæ growing from a well established stroma. Possibly rapid cork formation in the twig, occurring before the stroma is well established, would similarly have a protective effect.

Wiltshire (1915) observed the occlusion of the fungus by gum-like substances developed round the scab lesions. It has not been ascertained whether there are any varietal differences in its distribution.

In resistant varieties Wiltshire observed that the mycelium either failed to develop beneath the cuticle or shortly ceased to grow any further. A weaker development of mycelium has been observed by the present author in young leaves of the resistant variety Grenadier than in the susceptible seedling A. eleven days after inoculation (see Plates II. and IV., Part I., 1931).

Brooks (1928) thought this might indicate a lack of suitable food substances. The presence of toxic materials is also possible. With this in view Wiltshire made some preliminary experiments on the relative toxicity of liquids expressed from foliage of different varieties of apples on the germination of conidia of the fungus. He found that all the liquids expressed exhibited a certain degree of toxicity, but those derived from Bramley's Seedling and Newton Wonder (scab-resistant varieties) had a greater inhibiting effect on spore germination than liquids from King of the Pippins and Cap of Liberty (scab-susceptible). He concluded that a toxin might be present in greater quantities in the leaves of certain varieties and might exercise an important influence on the degree of resistance.

His work in this direction on the apple scab disease has not been extended until the present, but a brief summary of work on these lines in connection with other diseases may be cited before describing the details of the present investigation.

Walker (1924) showed that the varietal resistance of coloured varieties of onion to smudge was correlated with the presence in the outer scales of a flavone, absent from susceptible uncoloured varieties. Extracts obtained by steeping the scales in water were used as media for spore germination. Solutions from coloured scales inhibited or caused abnormal germination in the fungus spores, which germinated normally in the solution derived from susceptible colourless scales.

This point is interesting in connection with apple scab as Voges (1910) suggested that varietal resistance of Kalvill apples might be associated with red pigmentation of the skin, but Eriksson (1911) proved that such a theory was untenable.

Tims (1926) studied the effect of various extracts of the cabbage plant on the germination of the spores of *Fusarium conglutinans*, the causal organism of cabbage yellows. Inhibition was sometimes observed in extracts from resistant plants, but the results were not considered to give conclusive results. He thought, however, that the possibility of resistance being associated with a toxin was not entirely precluded, and suggested the importance of a sulphur glucoside in this connection.

Nobécourt (1928) obtained anomalous results from a study of the effects of plant extracts on spore germination of fungi incapable of parasitising the plant in question. They found no inhibition of spores of Botrytis cana, a parasite peculiar to *Allium cepa*, by extracts of various immune plant organs, such as lettuce leaves, Opuntia and broad bean extracts. Varietal resistance was not considered.

Brierley (1921) thought that the presence of a toxin in certain varieties of potato must be a factor in varietal resistance and susceptibility.

TECHNIQUE.

1. MATERIAL.

Following Wiltshire's procedure, expressed liquids were used in the present investigation as media for hanging drop cultures of scab conidia, controls in redistilled water being set up as controls.

Conidia from young leaf infections in the plantation were used when available ; otherwise conidia from culture, which were found to give qualitatively comparable results, were employed. Good crops of conidia were obtained on a thin layer of malt agar in petri dish, sown with a spore suspension in 1 c.c. of sterile water. The cultures were kept in an unheated incubator, and, after a short while, the aspect of the culture when the spores were mature was easily recognised.

Expressed liquids were obtained from various organs of the plant. The tissues were minced and pounded in a mortar and the juice usually expressed by squeezing the pounded mass between two pieces of wood in a vice. Later, silver discs, as used by Maskell (1927), were used to avoid any absorption of liquid by the wood. When using large quantities of material, a miniature cider press was employed and comparable liquids obtained. The pounded mass was placed in calico in the aluminium receptacle and the first oozings were discarded.

It is realised that the liquid obtained in this work is not equivalent in the proportion of its constituents to the cell sap within the plant. Hence the terms " sap " and " extract " are avoided. It is necessary to exercise caution in interpreting the results obtained with the expressed liquid, since it may not be absolutely comparable with the fluids affecting the fungus in the living plant.

2. HANGING DROP PREPARATIONS were made immediately after expression of the liquid so as to avoid initial variations due to enzyme action. No attempt was made to sterilise the liquid, chiefly owing to important changes in its effects caused by the action of heat.

By using sterile apparatus the development of contaminations during the period of germination was reduced to insignificant proportions. Where much contaminating matter appeared, the culture was rejected in making calculations.

Various methods of setting up the cultures were tried. It was essential for adequate aeration that the coverslips should be inverted, so that it was not possible to lay them in a damp chamber. Drops placed directly on the lids of Petri dishes proved unsatisfactory, as it was impossible to avoid a small amount of evaporation in some drops before the series was complete and water was very liable to condense among the drops. Excellent germination was obtained by spreading a spore suspension on agar in a Petri dish, as suggested by Mr. F. T. Brooks to the author. The effect of the liquid on the spores was not well shown, however, possibly owing to absorption of water from the agar causing dilution.

Ordinary van Tiegham cells were used for some time, but later the rings were enclosed in Petri dishes upon a layer of 10 per cent. gelatine, which was employed to preserve humidity in place of the usual drop of water. This method, suggested by Professor Barker, eliminated error caused by irregular condensation on the coverslip due to water in the cell. It was also more expeditious, as pains had not to be taken to ensure that the cells were adequately sealed, and further, each series of coverslips was maintained under similar conditions of humidity.

3. GENERAL OBSERVATIONS ON THE GERMINATING SPORES.

Wiltshire observed that germination of conidia in distilled water was sometimes not completed for 24 hours or longer. In the expressed liquid he noted varying degrees of inhibition of germination. Experiments by the present writer revealed that inhibition is not invariable in drops of the expressed liquid. Where a definitely toxic effect on germination was observed the following features were observed:

(1) retardation of germination. Some conidia became swollen, as occurs normally before germination, but the process advanced no further.

(2) abnormal germination. Certain conidia formed a transverse wall characteristic of the early stage of germination, but no germ tube was formed. Others put forth a germ tube, which tended to be very short and was often abnormally curved. Burst germ tubes, like those observed by Walker, were sometimes seen, chiefly in spores derived from pure culture.

(3) death of conidia. In some liquids the conidia appeared actually to be killed. They assumed a darker brown colour and became distinctly contracted.

Diagrams illustrating these features are appended (Figs. 1 and 2, Plate V.).

In liquids where germination was retarded, the spores began to grow more normally after a lapse of time. This indicated that the toxic effect of the liquid was gradually lost, and so it was necessary to observe the germination of the conidia after a definite length of time. The controls had usually germinated

PLATE V.

COMPARISON OF SPORES GERMINATING IN LIQUIDS EXPRESSED FROM LEAVES OF DIFFERENT VARIETIES.

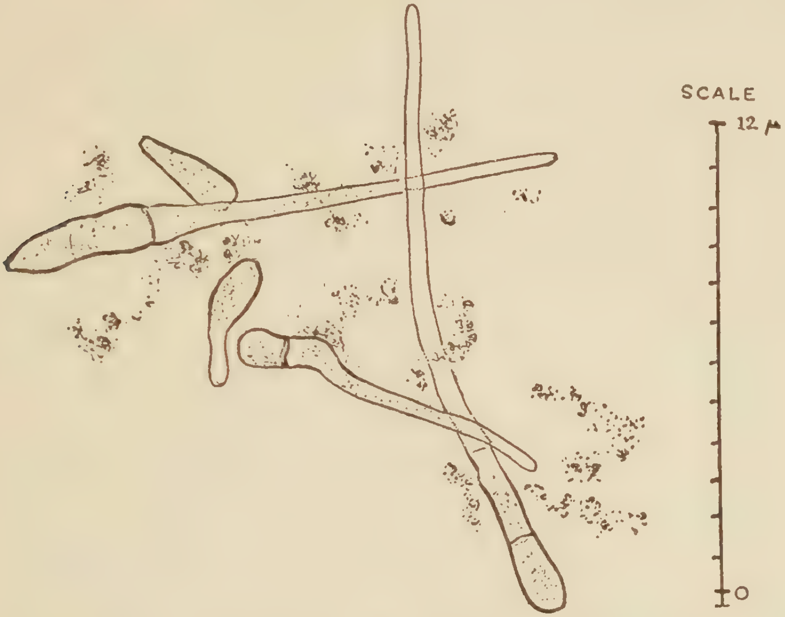


FIG. 1.

Spores from culture in liquid expressed from young leaves of Worcester Pearmain after 22 hours.

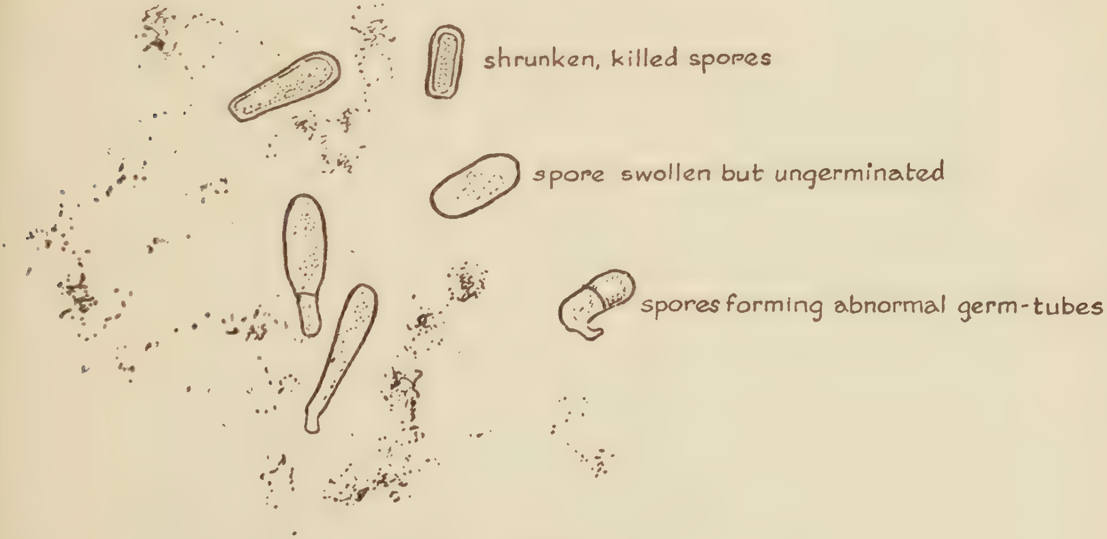

shrunken, killed spores
spore swollen but ungerminated
spores forming abnormal germ-tubes

FIG. 2.

Similar spores in liquid from Bramley's Seedling.

well after 18 hours and it was found convenient to make counts of all the cultures after 22 hours ; at this time the best comparison of the effects of different liquids could be made.

4. THE LIQUIDS STUDIED.

The effect was studied of liquids expressed from leaf, fruit and twig, special attention being paid to the leaf.

(*a*) *Liquids expressed from leaves.*

RESULTS.

It was originally hoped that a distinct germination percentage might be assigned to leaf liquids of each variety, indicating the relative toxicity of the sap. Preliminary experiments in the autumn of 1927 indicated that high germination occurred in liquids from susceptible varieties, while liquids from resistant varieties tended to inhibit germination more or less, but that a definite percentage correlated with varietal resistance was not likely to be obtained.

Work in 1928 and 1929 showed clearly that great irregularities existed in the toxicity of liquids expressed from young leaves of the same variety. Some of the factors associated with these irregularities were studied, and will be discussed later. In general the results warranted the conclusion that, when gathered under similar conditions, leaves from resistant varieties yielded liquids more toxic to the spores of *Venturia inaequalis* than leaves of the same age from susceptible varieties. The range of variation in most cases was wide, but the average germination in liquids from susceptible varieties was high and that in resistant varieties low.

Space will not permit a table of all the figures recorded to be given, but a list is given showing the average germination obtained for each variety, with the range of variation of toxicity appended. The numbers indicate the percentage germination of spores calculated as the percentage of spore germination in the controls (distilled water).

Some attempt was made to discover the causes of these wide variations and to find if they could be correlated with factors involved in the variation of resistance and susceptibility of the plant. The following factors will be considered in this connection:

i. Age of leaf.
ii. Diurnal variation in the toxicity of the liquid.
iii. Environmental conditions of the tree.
iv. Age of the tree and its fruiting or vegetative state.

i. *Age of leaf.*

In making comparisons of the toxicity of liquids, it was necessary to use leaves of similar ages. In general, young leaves almost fully expanded, but still light green in colour, were used.

TABLE VII.

Germination of spores of Venturia inaequalis in liquids expressed from young leaves of the apple.

Variety.	Observed susceptibility in the field.	Germination %(of controls).	
		Average.	Range of variation.
Worcester Pearmain ..	Very susceptible	66.91	99.26—29.54
Bismarck	,, ,,	(95.88)	
Morgan Sweet	,, ,,	55.61	110.77—14.48
Cox's Orange Pippin ..	,, ,,	120.88	147.3 —94.46
Allington Pippin	,, ,,	80.27	100.7 —53.04
Devon Quarrenden	,, ,,	(100.5)	
Cap of Liberty	,, ,,	56.34	81.16—31.52
Lord Suffield	Susceptible	50.06	88.34—13.99
Sweet Alford, cider orchard	,,	39.19	92.17—17.73
Sweet Alford, Nursery ..	Resistant	5.92	29.59— 0.0
Lane's Prince Albert ..	,,	(0.0)	
Crimson King	,,	26.23	64.0 — 3.04
King Edward VII.	,,	26.22	52.43— 0.0
Early Victoria	,,	18.37	42.13— 0.0
Charles Ross	,,	(30.0)	
Beauty of Bath	,,	(0.0)	
Newton Wonder	,,	38.36	69.69— 0.0
Bramley's Seedling	,,	10.77	32.98— 0.0
Dabinett	,,	24.024	51.21— 0.0
Grenadier	,,	8.92	17.84— 0.0

Results in 1927 and 1928 showed that a less toxic liquid was expressed from older leaves. In the following summer liquids were expressed from a series of leaves of successive ages from the Kingscroft Nursery at Long Ashton, where a number of leaves of equivalent ages could be obtained. Leaves of five ages were used, No. 1 being that just unfolded and No. 5 full sized and dark green in colour.

TABLE VIII.

Toxicity Variations according to Age of Leaf.

Date.	Variety.	Germination in controls.	Germination in liquids expressed from leaves.				
			Age 1	2	3	4	5
19.vi.29	Morgan Sweet ..	85.15	0.0		33.69	67.29	
1.vii.29	,, ,, ..	83.08	0.0	3.12	14.33	65.28	62.39
5.vii.29	Sweet Alford ..	65.10	0.0		0.0	0.0	20.30

Since the fungus is unable to establish itself in the mature leaf, these results appear to emphasise the disparity between the liquid expressed from a susceptible leaf and that encountered by the fungus beneath the cuticle. The liquid expressed from young leaves is much more turbid than that obtained from older leaves, so that the differences observed are an indication of increasing impermeability of the leaf.

Three times in July, 1928, contrary results were obtained. Liquids expressed from mature leaves of Lord Suffield (from Cheltenham) and a susceptible variety (from Flax Bourton) gave greater inhibition of germination than the liquid obtained from younger leaves.

ii. *Diurnal variation.*

The time of day at which the leaves were gathered was found to affect the toxicity of the liquid expressed from them. Some typical results are shown below in Table IX.

TABLE IX.

Diurnal Variations in Toxicity.

Date.	Variety.	Time of gathering.	% of germination.	
			In Controls.	In Liquid.
31.viii.28	Worcester Pearmain ..	10 a.m.	68.91	66.91
		3 p.m.	68.91	26.53
	Bramley's Seedling ..	10 a.m.	68.91	0.0
		3 p.m.	68.91	0.0
18.ix.28	Worcester Pearmain ..	8.30 a.m.	94.01	61.49
		3.30 p.m.	94.01	48.38
	Emneth Early	8.30 a.m.	94.01	13.99
		3.30 p.m.	94.01	0.0

General experience of liquids gathered at different times of the day, although not on the same date, confirms these results.

It seems probable that the greater toxicity of liquids expressed from leaves gathered in the afternoon is due to drying out of the leaves so that they yield a more concentrated liquid.

The possibility of alteration in composition due to photosynthesis and translocation was also considered. At 5 p.m. the petioles of certain leaves of the variety Morgan Sweet were covered with paraffin wax, a procedure which inhibits translocation from the leaf. Others were enclosed in sugar bags. Next morning liquids were expressed from the leaves with waxed petioles and from untreated leaves ; in the afternoon the leaves from bags and untreated leaves were used. The disadvantage of using the sugar bags was that evaporation was considerably lessened and the enclosed leaves had, at 3 p.m., the damp aspect of leaves collected in the morning. The results, given in Table X., afford some evidence that an increase in toxicity is associated with photosynthesis.

TABLE X.

Toxicity in Relation to Photosynthesis.

	% germination.	
	Leaves at 10 a.m.	Leaves at 3 p.m.
Normal leaves	83·77	39·64
Petioles waxed	80·35	
Leaves enclosed in bags		85·49
Controls	78·41	

Dilution of the expressed liquid results in a decreased toxicity analogous to that shown by liquids expressed from leaves in the morning, when their water content is greatest.

Wiltshire observed a gradual decrease in the toxicity of his expressed liquids by dilution with water, until at a proportion of 1 : 10 no difference from the water controls was seen. Owing to the great variation in the degree of toxicity of liquids no precise ratio can be expected. With Bramley's Seedling an increase was observed from a per cent. germination of 24.39 in an undiluted liquid to one of 56.18 by the addition of 3.5 c.c. of water to 7 gms. of leaves. Liquids expressed from Medaille d'Or fruits in November, 1927, lost their toxicity at a dilution of 1 : 6.

iii. *Nutrition of the tree.*

Considerable attention was paid in these investigations to the relations between the nutrition of the tree, its infection with scab and the toxicity of the liquid expressed from its leaves.

The general opinion of growers that nitrogen deficiency favours resistance suggested that exact observations in this field might be valuable. Little work has been done upon this aspect of pathology in fruit trees, but the relation of manurial treatment to varietal resistance of cereals to rusts has been studied by Spinks (1913), Stakman (1924), Hursh (1924) and others. Increased susceptibility is associated with high nitrogenous treatment and increased resistance with the application of potash. Stakman considers that the degree of physiologic susceptibility of different varieties of wheat to *Puccinia graminis* is not altered by nutrition, but greater damage occurs due to an increase of tissues liable to infection and a longer period of susceptibility. Salmon (1905) found that young leaves of *Hordeum sylvaticum* are susceptible to infection by conidia of wheat mildew, but that older leaves show a marked degree of physiological resistance.

Since the completion of this investigation at Long Ashton Mr. W. H. Moore (1930) has drawn attention to some observations made at East Malling in this connection. He noted that a series of " starved " trees were more heavily attacked by scab than a similar series receiving balanced manures, and also traced a differential effect of various rootstocks. Bramley's Seedling on Stock I., when manured was more heavily attacked than other stocks, but on unmanured plots showed the least infection in the Bramley series.

It has been mentioned already that in the case of apple scab there is some evidence for a shorter period of susceptibility in some nitrogen-deficient leaves, and possibly a longer period where excess nitrogen is present.

The observations made may be grouped as follows:

(1) observations of trees in grass and cultivated plots and under starved conditions.

(2) experiments on the inoculation of trees undergoing various nutritional treatments and in the toxicity of expressed liquids.

(1) *Trees in grass and arable plots.*

An experiment at Long Ashton, involving the grassing down of part of a cultivated plantation, resulted, by the summer of 1929, in a striking contrast between the trees. Those under grass showed rather yellowish leaves characteristic of high carbohydrate content, and those in the cultivated plots the dark green foliage associated with a high nitrogen supply. Some of the trees in the rows separating the two plots bore foliage of both types correlated with the different soil conditions on either side of the tree. The trees had been sprayed with Bordeaux mixture earlier in the season and consequently no material scab infection had occurred. Leaves were gathered and liquids expressed from the variety Blenheim Orange. Table XI. contains the results from the tests on these.

TABLE XI.

Grass and Arable Effects on Foliage Liquid Toxicity.

Source of liquid expressed.	% germ: of spores in liquid.
Trees in different plots:	
(1) cultivated plot (High N. ratio)	27.17
(2) grass plot (Low N. ratio)	0.0
One tree:	
(1) foliage from side over cultivated plot ..	67.47
(2) foliage from side over grass plot	6.28
Controls	92.28

These results indicate a striking difference between the toxicity of liquids expressed from foliage of the two types.

Some observations were also made on an old grass plot where the soil was found to be markedly deficient in nitrogen. In 1927 trees of Allington Pippin and Cherry Pearmain were more resistant to scab than trees of the same varieties grown under more normal conditions. No scab was found on Cherry

Pearmain (often a very susceptible variety) and on Allington Pippin relatively little, considering the wet season and that the trees had not been sprayed. A number of twigs of Allington Pippin were, however, attacked. No scab was observed in 1928. On the other hand, trees of both varieties under normal conditions were susceptible. Experiments on the leaf liquids indicated a corresponding toxicity.

(2) *Direct experiments upon the effect of various nutritional treatments.*

A series of pot cultures of Bramley's Seedling and Worcester Pearmain apple trees on the M. IX. rootstock was arranged by Mr. Wallace of this Station and placed at the disposal of the writer for inoculation with *Venturia inaequalis* and for experiments upon the expressed leaf liquids. The method in which the cultures were set up and the features shown by trees were the same as those described by Mr. Wallace (1925).

The toxicity of liquids expressed from young leaves of the same trees was tested at fortnightly intervals in the summer of 1929. The results obtained are shown below in Table XII. The upper figure denotes the germination of spores of the strain N. in the fresh unboiled liquid and the lower figure indicates germination in liquid which had been brought to boiling point and then cooled. Each number represents the mean per cent. germination of spores in the group of six hanging drop cultures in each series.

These figures, beyond showing a difference in toxicity between the two varieties, are not very strikingly significant ; probably complicating factors, whose differential effects on the several series could not be ascertained, obscure any definite results. Some indication of an increased toxicity associated with nitrogen deficiency is, however, suggested. In this connection the recent work of Pearsall (1928) is of interest. He observed a higher water content in several plants growing in highly nitrogenous soils. Hence any toxic substance present in the leaf of a tree receiving excess nitrogen might be expected to be rather more dilute in concentration than in a tree deficient in nitrogen and would be analogous in this respect to a leaf gathered in the early morning. The question of maturation would also be involved. Slow maturation, with the characteristic turbid liquid obtained from young leaves, was seen in nitrogen deficient leaves which might thus not be of truly comparable age (physiologically) with leaves from other series.

Inoculation experiments.

Inoculation experiments were made, parallel to the above, to test the toxicity of liquids expressed from leaves. These were also made at fortnightly intervals. The method of inoculation has already been described ; it must be noted that the use of glass lamp shades was not altogether satisfactory. A

TABLE XII.

Nutrition Deficiency Treatments in Relation to Toxicity.

Date : 1. Bramley's Seedling.	April 24	May 15	May 24	June 15	June 27	July 16	Mean
Controls (sterile water) ..	85.36	76.93	86.51	88.58	88.69	80.24	84.39
Series A .. .. .. (Complete nutrition) ..	5.48 20.67	0.0 12.22	25.47 38.43	0.0 8.44	0.0 37.09	0.0 15.7	5.33 22.09
Series B .. .. .. (Nitrogen deficient) ..	0.0 21.38	0.0 1.39	0.0 9.69	0.0 15.32	0.0 20.25	—	0.0 13.60
Series D .. .. .. (Potash deficient)	46.16 45.13	3.09 29.72	0.0 16.67	0.0 7.02	0.0 23.38	0.0 24.59	8.37 24.4
Series E .. .. .. (Phosphate deficient) ..	65.14 51.37	0.0 1.39	0.0 39.98	0.0 22.27	0.0 16.21	0.0 23.47	10.45 25.78
Series F .. .. .. (Calcium deficient) ..	42.58 39.85	2.38 0.0	0.0 0.0	0.0 0.0	0.0 34.14	0.0 20.52	7.45 15.75
Series G .. .. .. (Magnesium deficient) ..	26.63 44.61	2.5 25.48	0.0 0.0	0.0 17.72	0.0 0.56	0.0 18.81	4.85 17.87
Series H .. .. .. (Rain water only)	0.0 63.25	0.0 4.14	0.0 0.0	0.0 10.87	11.29* 8.56	—	 17.36

* Experimental error suspected.

2. Worcester Pearmain.	April 24	May 15	May 24	June 15	June 27	July 16	Mean
Controls	84.27	83.0	95.21	75.08	85.75	75.61	83.15
Series A	83.66 75.16	45.26 41.91	70.17 46.67	22.19 8.19	51.81 29.05	71.22 33.56	57.39 39.09
Series C	71.85 63.69	40.45 30.62	68.92 23.22	5.59 10.56	38.94 33.77	30.40 26.23	42.69 31.35
Series D	73.14 79.15	67.14 59.12	84.93 62.79	31.12 16.11	60.86 25.08	58.24 19.93	62.57 43.69
Series E	83.15 79.61	58.61 46.57	68.11 39.94	31.09 12.61	74.81 30.33	52.51 24.23	61.38 38.88
Series F	81.08 67.54	64.25 48.41	32.96 56.69	24.16 0.0	58.91 27.83	56.29 14.12	52.94 35.77
Series G	86.76 74.45	73.32 38.47	75.97 66.86	37.22 15.64	72.32 32.63	31.76 15.05	62.89 40.50
Series H	87.53 78.61	37.84 47.06	64.78 48.01	22.01 0.0	74.48 25.29	41.58 20.91	54.70 36.65

certain amount of overheating could not be avoided ; this was definitely injurious to the fungus and sometimes caused scorching of the leaf. It also appeared to promote dropping of the fruits. Conidia from leaves of Morgan Sweet (strain N) were used, when ascospores were no longer available.

The results of the experiments are shown in Diagram II. The date of inoculation is indicated by a cross. The appearance of a spore-bearing lesion is shown by a continuous line, a dotted line indicates a very small lesion or one becoming moribund.

Some features of interest are revealed by the following analysis:

TABLE XIII.

Infection Trials under Nutrient Deficiency Conditions.

Variety and Nutrition.		No. of Inoculations.	No. of infections.	Average length of incubation (days).	Average persistence of individual lesion (days).
Bramley's Seedling	A.	8	6	31.83	23.25
	C.	7	2	27.5	9.0
	D.	8	4	26.0	39.25
	E.	8	3	27.0	22.0
	F.	8	4	30.0	24.5
	G.	8	5	23.8	25.0
	H.	8	2	27.5	7.0
Total	..	55	26		
Mean	..			27.59	21.43
Worcester Pearmain	A.	8	7	24.14	27.0
	C.	7	4	25.75	13.5
	D.	8	8	25.0	47.14
	E.	8	4	24.25	25.5
	F.	8	5	23.25	32.25
	G.	8	5	25.6	31.8
	H.	8	1	19.0	1.0
Total	..	55	34		
Mean	..			23.80	26.88

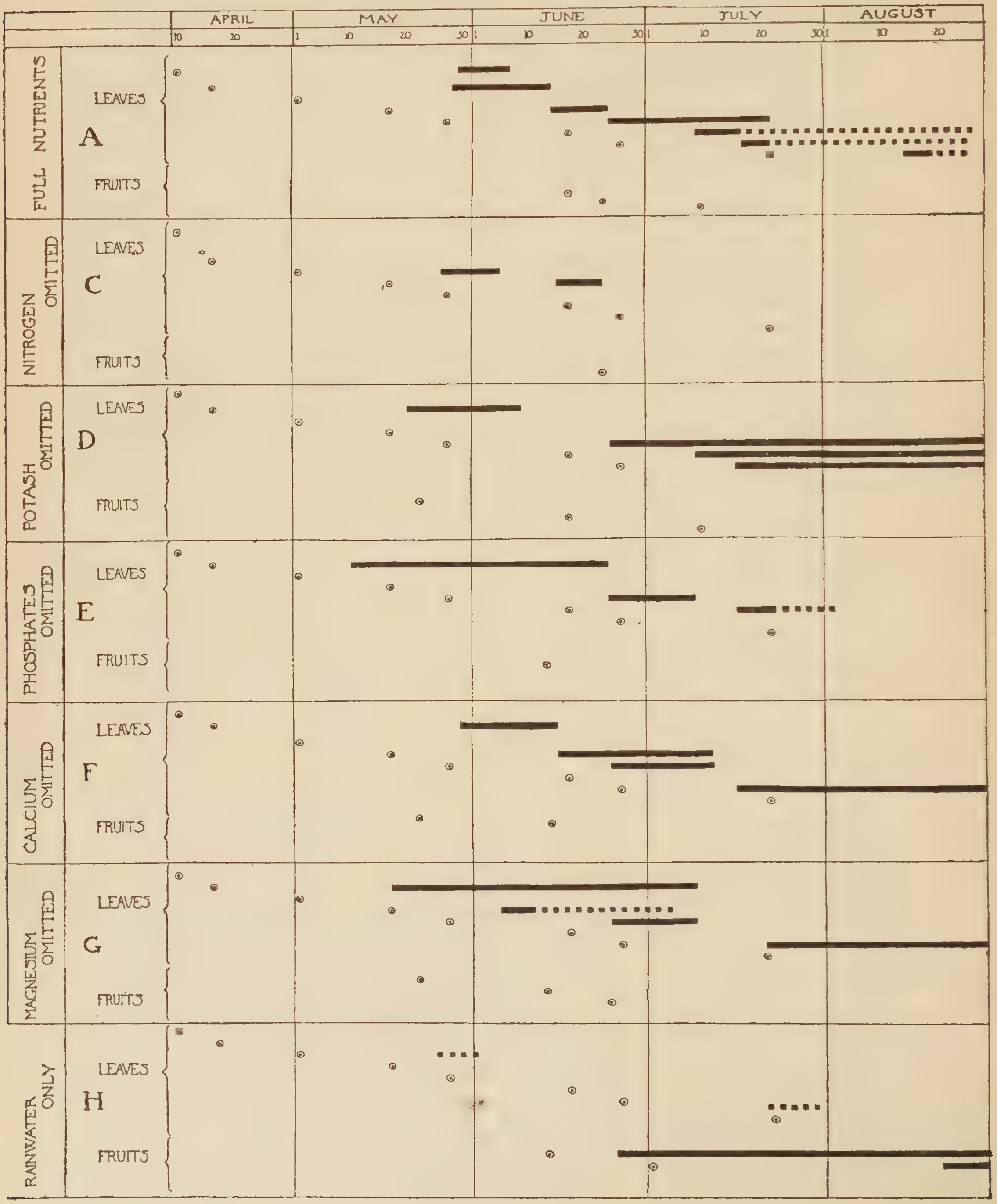
Features worthy of notice are:

(1) The period of incubation was, on the average, longer in Bramley's Seedling than in Worcester Pearmain, but showed no relation to the nutrition of the tree. It varied considerably and was affected by weather conditions.

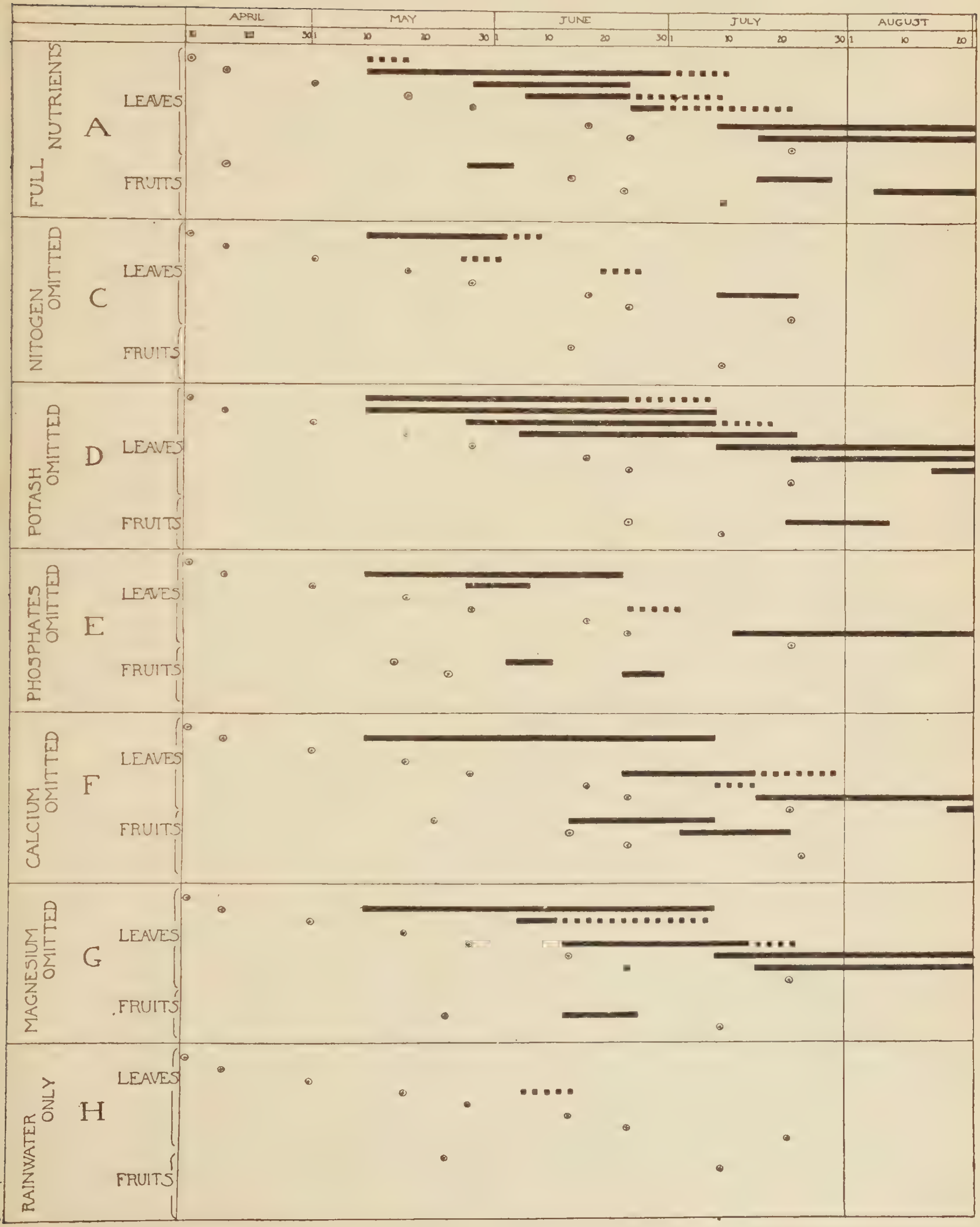
(2) A definitely lower proportion of infections occurred in the nitrogen-deficient series, and that receiving rainwater only, in both varieties.

(3) Variation can be observed in the time of persistence of individual lesions in a spore-producing state on the leaf. In general, persistence was shorter in Bramley's Seedling than in Worcester Pearmain. It was remarkably

RESULTS OF INOCULATIONS OF APPLE TREES GROWN UNDER DIFFERENT NUTRIENT CONDITIONS I BRAMLEY'S SEEDLING



II WORCESTER PEARMAIN



short in series C and H, markedly larger in series D and somewhat longer in F and G.

(4) There was no apparent change in the resistance of a variety of series during the season.

A consideration of these results leads to the conclusion that the nutrition of the tree definitely affects its susceptibility to scab infection. The fungus develops less extensively and causes less injury on trees markedly deficient in nitrogen.

Fewer infections probably indicate the presence of toxic substances in the leaf, but the small size of lesions and their early disappearance suggests that the fungus may be unable to obtain substances, possibly nitrogenous in character, suitable for its own nutrition. It may be mentioned in this connection that some microscopical preparations indicated that the cuticle was subnormal in thickness when the tree was deficient in nitrogen. Any toxic substances present in the cuticle were thus liable to be more concentrated ; also less cuticle was available as a source of food material.

It is probable that the fungus is capable of obtaining nutrition from the cuticle as subtratum. Some pieces of cuticle of the fruit of Bramley's Seedling were boiled in potash, sterilised and placed in sterile distilled water. These were inoculated with *Venturia inaequalis* from pure culture in January, 1929. The fungus grew and had produced a few spores in September, 1929.

It is also worthy of note that the foliage characters associated with potash deficiency in cereals, i.e., luxuriant, soft leaves, are not characteristic of potash-deficient apple trees, where the leaves are subnormal in size. Hence any increase in susceptibility of potash-deficient trees must be associated with physiological, rather than morphological characters. However, nitrogen excess associated with calcium and magnesium deficiency is characterised by large foliage, which is rather more susceptible to infection.

When pathological features appeared in the leaves, e.g., bronzing in series E and blotching in series G, scab lesions rapidly died off in the affected areas.

iv. *Age of tree and its vegetative and flowering condition.*

In some nursery stock a greater resistance to scab was observed than in orchard trees of the same variety. For example, young trees of Sweet Alford in the Kingscroft Nursery at the Station were practically free from scab in 1928 and 1929, while trees of the same variety in the cider orchard were considerably infected. The question of specialised strains has already been considered in this connection. On the other hand, scab is often prevalent among young trees which have not yet reached a flowering state.

These two points are mentioned, since a high nitrogen content is recognised as characteristic of the vegetative tree and high carbohydrate content of the flowering tree. If differences in manurial treatment are important, it would appear possible that these conditions would also affect resistance, but so far no clear relation has been observed.

(*b*) *Liquids expressed from the fruit.*

Liquids were obtained by expressing pounded masses of the tissues of the fruit in the same way that liquids were expressed from leaves.

In the autumn of 1927 a number of fruits were tested thus and, except for the variety Cap of Liberty, none yielded a toxic liquid, although resistant to scab. A few varieties, e.g. Medaille d'Or and Allington Pippin, revealed greater toxicity after storage.

Experiments made on the fruits in 1928 showed that liquids from the young fruits were extremely toxic. In June, 1928, liquids from the varieties Sweet Alford, Cap of Liberty, Cherry Pearmain, Medaille d'Or and Dabinett, all inhibited spore germination completely. In July the liquid from Sweet Alford had lost its toxicity and allowed high spore germination for the rest of the year. Although variable, liquids from Cap of Liberty, Dabinett and Medaille d'Or remained on the whole toxic.

These two features of the liquids are worthy of discussion.

(i) *Variable effects of fruit juices on spore germination.*

The effects of the fruit juices obtained varied greatly in toxicity in the two seasons and no correlation of toxicity with varietal resistance can be discerned. For example, the juice of Cap of Liberty (a susceptible variety) has been very toxic throughout the study, while that of Bramley's Seedling (resistant) often allowed high spore germination. Expressed juice of the fruit may be very different, however, from fluids with which the fungus would come in contact beneath the cuticle. Not only do changes occur during expression, but the structure of the fruit indicates that the tissues in the skin would have quite a different chemical composition from the deeper seated tissues from which the juice is chiefly derived.

(ii) *Liquids from young fruits.*

Complete inhibition of spore germination was caused by all fruit liquids until July, in the summer of 1928. As just mentioned, as the process of ripening set in, marked by the appearance of sugars, toxicity disappeared in Sweet Alford, but continued in Medaille d'Or and Cap of Liberty and only showed a slight decrease in Dabinett. It is thought that this phenomena is related to the production of toxic tannin-like substances by wounding, as described by Cook and Taubenhaus (1911). They found that on wounding unripe pomaceous fruits, these toxins were produced by the action of an oxidase on a polyatomic phenol. As maturity approached, oxidase activity lessened and greater susceptibility to various rots was observed. Hence it is possible that fruits such as Sweet Alford, low in astringency, might lose this toxin-forming capacity, but in others of high astringency the action might be more likely to persist.

Susceptibility to scab, however, is a character of the young fruit in contrast to the mature apple ; but penetration by the fungus does not involve wounding of the epidermal cells, or production of the tannin-like toxins. For successful infection a nicely balanced reaction between parasite and host is necessary, resembling that postulated by Kharbush (Nobécourt, 1928) for rust infection of cereals. The parasite must be able to grow sufficiently vigorously to obtain its nutrition from the host, and yet not to injure the substratum enough to cause toxin formation.

In this way fruit infection appears to differ from leaf infection. Extensive necrosis of the host cells is frequently observed in the leaf, but in the fruit injury is chiefly due to pathological changes in the nature of cork and gum formation.

The work of Drabble and Nierenstein (1907) on the close association of the tannins with phellogen formation is interesting in this connection. It has previously been remarked that rapid cork development is thought to prevent the parasite from becoming well established. Hence stimulation to profuse tannin production might lead to early and complete occlusion of the parasite, as in Medaille d'Or. Less tannin production, associated with or accompanied by more irregular phellogen, results in incomplete isolation of the fungus. Miss Tetley (1929) has described such irregular phellogen development in Cox's Orange Pippin, a very susceptible apple.

(c) *The presence of " tannins " in the expressed liquid.*

A few experiments were performed to ascertain whether the toxicity of certain leaf and fruit extracts was in any way associated with the presence of " tannins." The term " tannin " is here employed to include all astringent substances precipitated by gelatine and giving the colour reaction with ferric chloride characteristic of the tannin group.

1. REMOVAL OF TANNINS.

Some preliminary experiments on the removal of tannins were performed in December, 1928, and some typical results are shown in Table XIV. Tannins were removed by precipitation with gelatine. A solution of such concentration that only a drop or two had to be added prevented dilution effects from interfering. In some cases it was possible to remove the gelatine-tannin precipitate by filtering ; in others the insoluble precipitate was included in the culture.

TABLE XIV.

Effects of Removal of Tannins on Toxicity of Liquids.

Date.	Variety.	Treatment of liquid.	Spore germination.	Controls.
6.xii.28	Cap of Liberty ..	Untreated ..	0.0	65.11
		Gelatine ppt. removed	57.81	
	Dabinett	Untreated ..	0.0	
		Gelatine solution added	55.07	
7.xii.28	Bramley's Seedling	Untreated ..	9.33	74.65
		Gelatine solution added	48.52	
14.xii.28	Bramley's Seedling	Untreated ..	26.89	62.55
		Gelatine solution added	72.5	
	Frequin d'Audievre	Untreated ..	50.48	
		Gelatine solution added	54.97	
	Medaille d'Or ..	Untreated ..	0.0	
		Gelatine solution added	0.0	
		Gelatine ppt. removed	25.91	

Some toxic principle was evidently removed by the gelatine in these varieties, but it is noteworthy that the untreated juice from Bramley's Seedling (low astringency) is more toxic than that of Frequin d'Audievre (high astringency). In the variety Medaille d'Or, filtration had a distinct effect.

In 1929 some similar experiments were made on liquids derived from leaves. For comparative purposes, certain portions of the liquid were diluted

TABLE XV.

Date.	Variety.	Treatment of liquid.	Spore germination.	
			Liquid.	Controls.
26.vii.29	Bramley's Seedling	Untreated liquid ..	26.53	64.31
		Untreated liquid (diluted)	22.24	
		Untreated liquid (diluted and filtered)	54.81	
		Liquid + gelatine ..	51.15	
		Liquid + gelatine (filtered)	63.66	
23.vii.29		Untreated liquid	32.24	70.67
		Untreated liquid (filtered)	44.14	
		Untreated liquid (diluted)	48.15	
		Liquid and gelatine ..	53.01	
		Liquid and gelatine (filtered)	66.97	

with equal volumes of water, so that the effects of dilution and the addition of a few drops of gelatine solution could be compared. For results see Table XV. appended.

These results suggest that reduced toxicity results from three different treatments (1) filtration, (2) dilution, (3) addition of gelatine.

(1) *Filtration.* In the crude extract a large amount of cytoplasmic fragments and cell inclusions are present, so that on standing certain moderately soluble substances would gradually come more into solution. The effect of filtering the liquid immediately after expression indicates that certain of the toxic substances are of this sparingly soluble nature.

(2) *Dilution.* It has already been noted that an increase in the water content of the leaf, or a dilution of the toxic substances, leads to reduction in the toxic effects of expressed liquids on the germinating spore.

(3) *Addition of gelatine.* Since the volume of gelatine solution required to form a precipitate is so much less than that of the volume of water added in diluting the liquid, a definite reaction of the gelatine with a toxic substance must be postulated. Whether the compound is liable to affect the spores, or whether the result of subsequent filtration represents the combined effect of gelatine and filtration, there is no evidence to decide.

Leaching. Leaves of Morgan Sweet and Sweet Alford were leached with cold water in the manner described by Mann and Wallace (19). Substances giving a colour reaction with Ferric chloride came into solution, indicating that astringent substances are liable to pass outwards through the cuticle. The fungus growing beneath the cuticle is thus liable to be affected by them. Germination of spores was found to be equally good upon resistant and susceptible foliage ; there is no possibility of a toxic substance being deposited on the leaf surface by leaching.

Staining. Sections of the skin of the storage apples used in the experiment described were stained with nitrous ether in the manner described by Vinson (1910). Abundant " tannins " were present in the epidermal cells and the cuticle itself showed a faint umber colour.

Leaves fixed in a chrom-acetic fixative also showed a rich tannin content in the epidermal cells ; the cuticle was quite colourless after the use of this fixative. No varietal differences were observed.

Tannic acid. Although this acid is without direct relation to leaf " tannins," its toxicity to *Venturia inaequalis* is of interest in this connection.

The following data were obtained:

TABLE XVI.

Effect of Tannic Acid Solutions on the Germination of Conidia.
Actual Percentage Germination.

.50%	tannic acid	0.0					
.25%	,,	0.0	0.0				
.1%	,,	0.0	0.0				
.05%	,,	0.0	0.0				
.03%	,,				0.4	0.0	2.02
.025%	,,	0.0	7.78	24.00	30.79	0.0	5.0
.02%	,,				19.59	7.13	18.85
.015%	,,					5.92	23.48
.01%	,,	47.09	42.06	88.55	39.99	37.55	67.59
.005%	,,	75.37					
Controls		81.34	67.96	91.09	74.51	54.17	75.39

A graphic representation of these results is shown by graph I. For comparison it was observed that 0.5 per cent. tannic acid had no effect on germination of Fusarium spores of *Nectria galligena,* Bres., *Penicillium* sp. and various yeasts.

Besides showing the susceptibility of *Venturia* to small quantities of tannic acid, these results show the limits of accuracy which can be expected in work of this kind. Variations might be due to differences in vigour of the spores, and differences in moisture of their walls. A substance of toxicity equivalent to that of tannic acid might vary to 0.02 per cent. without a significant change being observed.

(*d*) *Enzyme action in the expressed liquid.*

Enzyme action taking place in the liquid after expression affected considerably its toxicity for the fungous spores. The following aspects were studied:

(1) Loss of toxicity on keeping the liquid ; or " staling."

(2) Effect of heat in destroying enzymes.

(3) Effect of alcohol in removing enzymes.

(1) *Staling.* Wiltshire (1914) found that the expressed liquid gradually lost its toxicity on keeping. Further observations have been made by the present writer.

The staling process in the liquids was estimated by observing spore germination after successive periods of time had elapsed and also by making new cultures from the original liquid, so as to compare the change in toxicity both in the presence and absence of the spores.

Some of the results are shown graphically in graphs III., IV. and V., which are given as examples from a number of similar curves obtained, for the varieties Sweet Alford, Morgan Sweet, Dabinett, Cap of Liberty, and King Edward VII. The continuous lines indicate the changes in germination percentages in the hanging drops, the dotted line represents the staling of the liquid in the tube.

Rather more rapid loss of toxicity in a tube of King Edward VII. liquid kept at 25° C., previous to culturing, than in that at 18° C., indicated a greater velocity of enzyme action at the higher temperature. Some additional figures show that in very young leaves a significant loss of toxicity occurs after keeping the liquid for four hours.

Staling in the liquid expressed from the ripe fruit of Cap of Liberty was also observed, and is shown in the graph. Here it is noteworthy that practically no staling occurred in the absence of the spores, while it occurred much in the same way as in leaf liquids in the hanging drops. This suggests that no toxin-removing enzyme is present in the ripe fruit of Cap of Liberty, but that such an enzyme may be produced by the germinating spore, and exists in the liquid derived from the leaf. On the other hand, the staling of the boiled fruit juice was slower than that of the fresh liquid ; possibly a greater amount of toxin was developed by boiling, as will be suggested later.

In general, spores are often killed outright by toxic substances in the expressed liquid, so that another factor complicating the significance of spore germination is introduced. In a liquid of high toxicity the dead spores would be observed after 22 hours, but in one of toxicity insufficient to kill them the effects of enzyme action would become apparent within 22 hours and the spore germination would not be comparable to that of the highly toxic liquid.

(2) *Destruction of enzymes by heat.*

Experiments on staling have already shown that toxin-destroying enzymes are inactivated by heat. Experiments made in the spring of 1928 and in 1929 show the great increase in toxicity caused by heating the liquid to 70-80° C. or more before using it as a culture medium. Graphic representations indicate the results most clearly and show the characteristic curves obtained. It is noteworthy that the effect was observed in both diluted and undiluted liquids. See Graph II.

Later in the season much more variable results were obtained. As a rule greater inhibition was observed in boiled, compared with unheated liquids, particularly in 1927 and 1928. In 1929 it was noted that in liquids expressed from young leaves of Worcester Pearmain, Sweet Alford, Dabinett and Cap of Liberty during the first week in May, spore germination was moderately high, but was completely inhibited after the liquid had been boiled. Young leaves from the same trees later in the season yielded liquids whose toxicity was decreased by boiling. With adult leaves the effect of heat was very irregular. The sudden change to complete inhibition after boiling was never observed ; increase and decrease in toxicity by boiling were equally frequent.

Dried leaves. A few experiments were made in this connection with leaves dried in air in a greenhouse (when the enzymes would be uninjured), and in an oven at 100° C. (when the enzymes would be destroyed). The dried leaves were extracted with the volume of water lost on drying and the toxicity of these liquids tested. The results were rather unexpected. Liquids derived from leaves dried in air showed a high degree of toxicity, while this liquid after boiling became less toxic ; leaves dried in the oven yielded a liquid of low toxicity.

(3) *Removal of enzymes.* An attempt was made to remove enzymes by precipitation with alcohol and compare the liquid with that in which enzymes had been destroyed by heat.

An undiluted expressed liquid was measured and filtered. To the filtrate 90 c.c. of methylated spirit was added and the precipitate removed by filtering. The filtrate was evaporated at 40-50° C. on a water bath under reduced pressure and washed twice. It was reduced to rather less than its original volume and the correct quantity regained by dilution.

The chief difficulty was that of obtaining a liquid at the end of the experiment exactly equivalent in concentration to that originally expressed. The results are shown in Table XVII.

TABLE XVII.

The Effects of Enzyme Removal on Toxicity.

Treatment of Liquid.	Germination of Spores.		
	Variety : Sweet Alford. 7.vi.28.	Variety : Devon Quarrenden. 16.v.28.	Variety : Bramley's Seedling. 11.v.28.
Untreated liquid, unheated	59·81	40.15	37·04
,, ,, boiled	0.0	1.84	0.0
Alcohol p.p.t. removed, unheated	3·68	20.57	14·94
,, ,, heated to 70°C.	0.0	0.0	14·89
,, ,, heated to 80°C.	0.0	0.0	12.68
,, ,, boiled	0.52	0.0	6·49
Untreated liquid—liquid less enzymes	43·43	42.19	
Crude filtrate added to liquid, less enzymes	44·32	14·37	
Controls	92.70	60.00	81.69

Though inconclusive, these results suggest that a toxic substance present in the freshly expressed liquid is gradually removed by the action of enzymes.

When these are removed by alcohol, a toxicity is recorded equivalent to that of the liquid immediately after expression.

Acidity of the juice.

No correlation was observed between toxicity of the liquid and its pH concentration. The pH concentration of both leaves and fruits was found to vary between an acidity greater than pH 3.0 and pH 5.5. This is well within the range of acidity tolerated by the fungus.

The action of heat.

Experiments on the removal of enzymes indicated that a change of toxicity may be caused by the action of heat apart from inhibition of enzyme action. Some such reaction as the hydrolysis of glucosides in the presence of acids was thought to be possible, especially as the process occurred readily in diluted liquids and in young leaves where such substances would be easily extracted.

Some experiments were made to discover whether the effect of boiling was associated with the presence of acids. Liquids were neutralised and boiled, and reacidified and boiled and the effects of boiling in the presence of acids deduced.

These results, though rather inconclusive, indicate that in some cases, e.g., leaves of Warner's King and fruit of Sweet Alford, the presence of acids caused a greater increase in toxicity on boiling. At least two factors may be involved in determining the differences between the toxicities of boiled and unboiled liquids ; the toxin-destroying enzymes may be inactivated and a higher concentration of the toxin may be evolved by heating in the presence of acids. The reversible nature of enzyme action difference in the physiological state of the leaf would result in different relations between the boiled and unboiled liquids.

Since the metabolism of all plants, including the tree and the fungus, depends upon reactions governed by enzymes, it is significant that a study of varietal resistance of a higher plant to a fungus parasite should emphasise the importance of the inter-relations of the enzyme systems of the two organisms. Several investigators, in particular Klotz (1928), have observed that liquids extracted from closely related varieties of a host plant inhibit the velocities of fungal enzymes in a manner correlated with varietal resistance. The action of enzymes in the host must also be important ; antagonism or harmony of the two systems is probably one of the most important factors in resistance and susceptibility to disease.

SUMMARY—PART II.

1. Barriers of an anatomical nature are considered to be of subsidiary importance in determining varietal resistance of the apple to scab.
2. Previous work on the relationship between the effects of expressed liquids on spore germination and varietal resistance is mentioned.

3. A description is given of the technique employed for studying spore germination in expressed liquid and general observations on the cultures.

4. Within a wide range of variation, leaves of resistant varieties yielded a more toxic liquid than those of susceptible varieties.

5. Variation in the toxicity of a liquid from the same variety was found to be affected by the age of the leaf, the time of day at which it was gathered and the nutrition of the tree.

   (i) A more toxic and apparently more highly concentrated liquid was obtained from young leaves.

   (ii) The liquid was more toxic when expressed from leaves gathered in the afternoon.

   (iii) Highly toxic liquids were obtained from trees suffering from a deficiency in nitrogen.

6. Parallel experiments made in inoculating trees under various nutritional conditions revealed that the period of incubation was longer in Bramley's Seedling than in Worcester Pearmain, but showed no relation to the nutrition of the tree. A lower proportion of infections occurred in trees of both varieties which were deficient in nitrogen or received rainwater only. Persistence of individual lesions was shorter in Bramley's Seedling than in Worcester Pearmain. It was remarkably short in trees deficient in nitrogen, markedly longer in trees deficient in potash and somewhat longer in trees deficient in calcium and magnesium.

7. Liquids expressed from fruits varied in their toxicity ; those from young fruits were highly toxic.

8. A toxic substance, possibly of an astringent nature, was removed by the addition of gelatine to the liquid and by filtration. The remarkable toxicity of tannic acid to *Venturia inaequalis* is mentioned.

9. The toxicity of the liquid is modified by enzyme action after expression.

   (i) A loss in toxicity is observed when the liquid is kept for some hours.

   (ii) Liquids in which the enzymes are destroyed by heat or removed by alcohol show, in general, greater toxicity than fresh extracts.

10. Toxicity of the liquid shows no relation to its pH concentration, but possibly the toxin is increased by boiling when the medium is acid in reaction.

GENERAL DISCUSSION.

Parts I. and II.

The general conclusion to be drawn from the evidence obtained from this work would seem to be that the ultimate character distinguishing a resistant from a susceptible variety of the apple is associated with the nature of the fluids bathing the epidermal cell walls which form the environment of the parasite. In a susceptible variety the fungus is able to establish itself beneath the cuticle since a congenial environment is encountered ; in a resistant variety the environment is uncongenial.

A study of spore germination and penetration, described in Part I. of this paper (1931), suggests that no factors associated with these processes are sufficient in themselves to distinguish resistant and susceptible varieties. Differences in the frequency with which scab is observed on certain varieties is, however, dependent on some morphological characters of the host. For example, protective hairs reduce the liability of an organ to infection by eliminating germinating spores. Morphological characters (changing with increasing age) in the cuticle result in differences in the length of the period of susceptibility of some susceptible and resistant varieties.

The distribution of scab must also be affected by the relative abundance of fungous spores in the orchard and their viability. The presence of a virulent strain or one suitably specialised biologically are also found to be important.

True resistance, most satisfactorily estimated by direct inoculation experiments, seems to depend on the physiological relations between host and parasite within the leaf. In the present part (Part II.) of this paper an account has been given of the effect of liquids expressed from leaves and fruits on the germination of spores. It is fully realised that the technique employed is crude and no attempt is made to make a direct comparison between the liquids expressed and the sap within the living organ. This is specially applicable to liquids expressed from mature leaves and fruits.

A large number of experiments upon liquids expressed from young leaves has given the general result within a wide range of variation that liquids derived from resistant varieties are constantly more toxic to the spores than liquids from susceptible varieties. Experiments made upon trees of the same variety undergoing different nutritional treatment show a similar relationship of toxicity with infection. Since comparison was made with controls in distilled water, these results support the view that failure of the fungus to grow beneath the cuticle is due to toxicity of the sap rather than to an absence of suitable nourishment. Several attempts by other investigators to obtain substances toxic to rusts from resistant varieties of cereals have yielded negative results ; varietal resistance of the apple to scab may thus be in contrast to varietal resistance of cereals to rusts.

Features of infections on nitrogen-deficient trees suggest, however, that some starvation phenomena may also be involved in such trees.

Toxicity of the liquid is rapidly modified by enzyme action after expression, but it is impossible to correlate this with enzyme action in the organised sap. The phenomenon emphasises, however, the importance of compatible enzyme systems of host and parasite for infection by the fungus to be established.

No definite constituent of the liquid has been defined as the toxin. Although no correlation with the total content of " tannins " (or astringent substances) and varietal resistance can be traced, experiments indicate that *Venturia inaequalis* is readily injured by such substances present in the liquid. Possibly a substance of this nature is formed during metabolic changes in the leaf, the degree to which the fungus is affected being modified by enzyme activity of both host and parasite.

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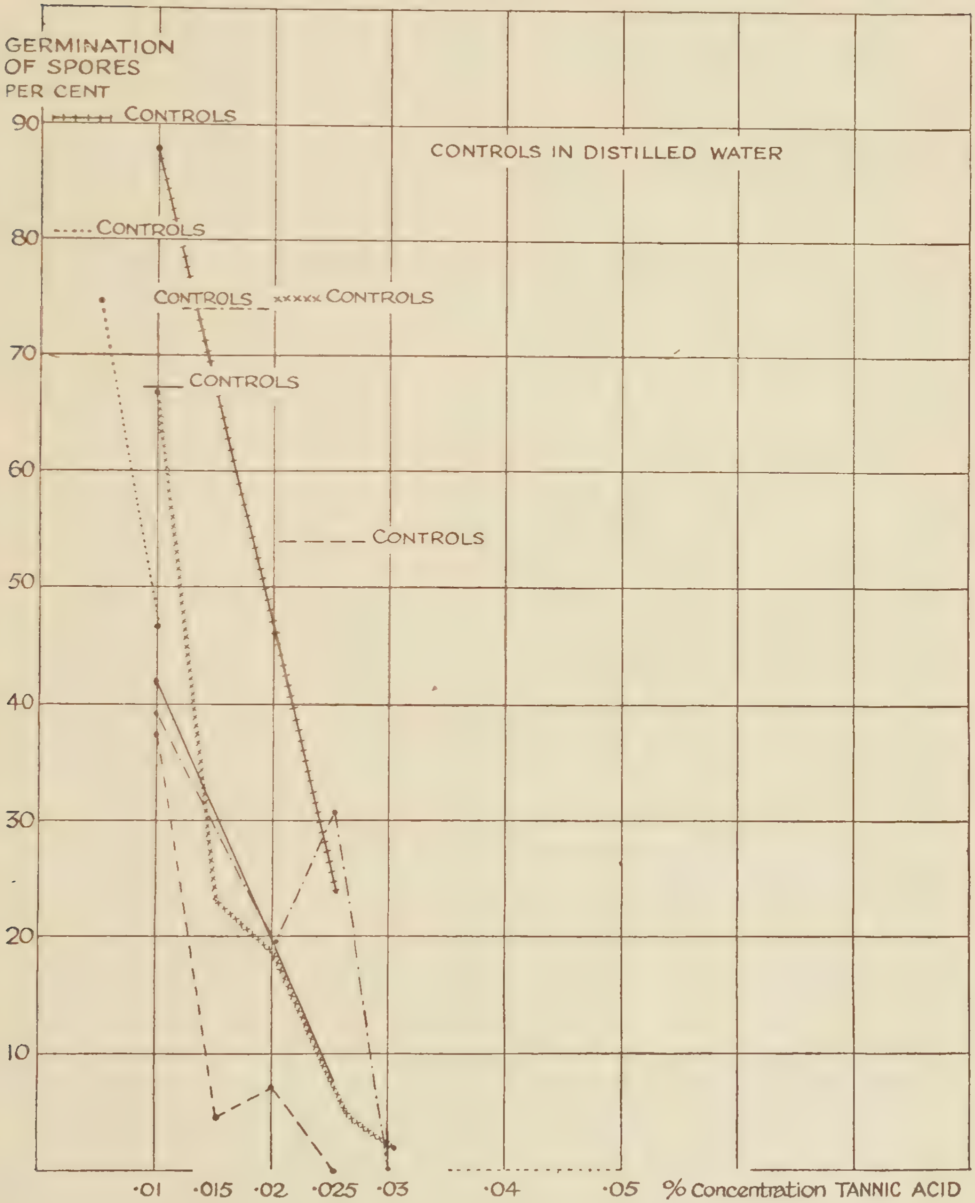
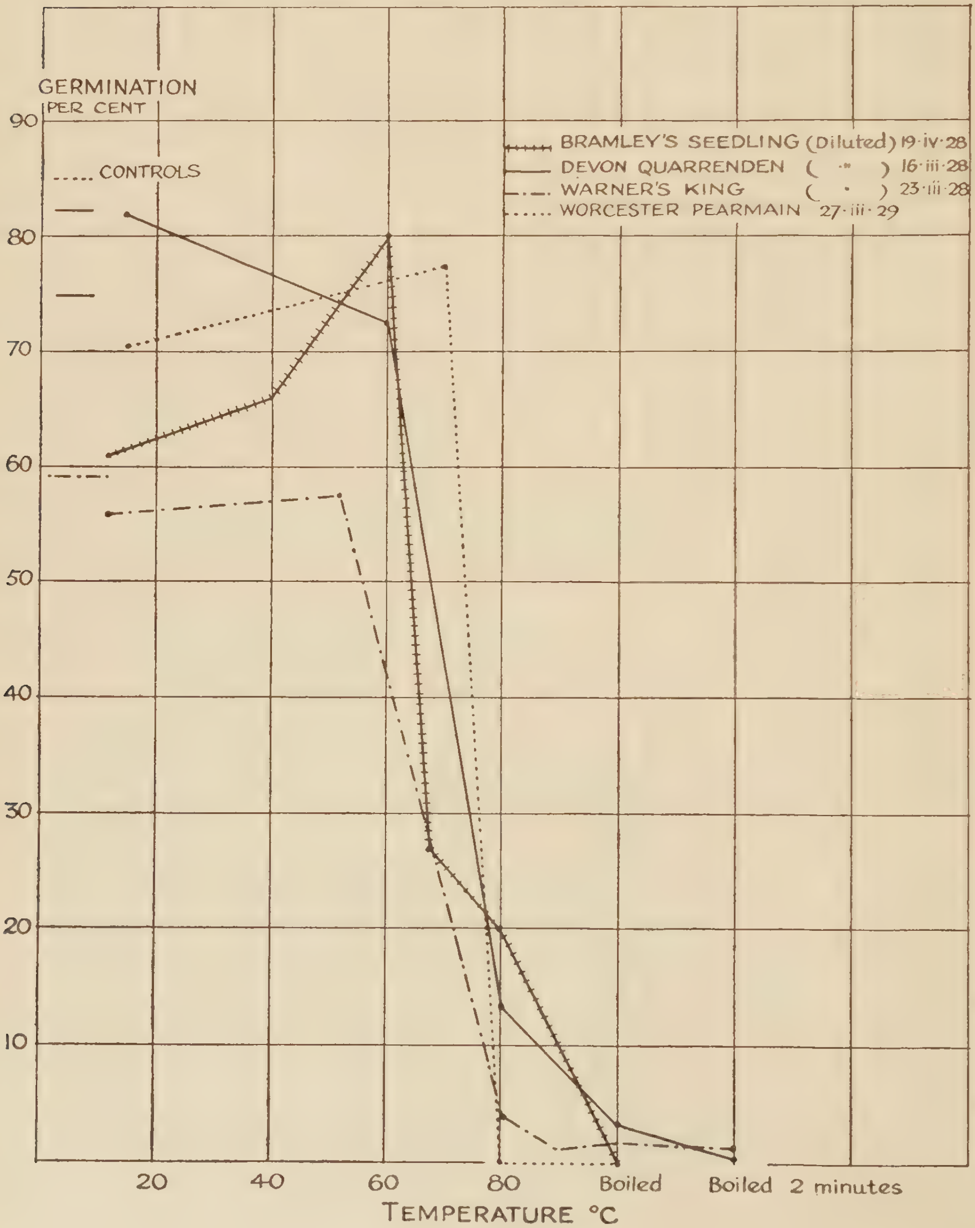
GRAPH I.

THE EFFECT OF SOLUTIONS OF TANNIC ACID ON THE GERMINATION OF CONIDIA OF *VENTURIA INAEQUALIS*.



GRAPH II.

GRAPH ILLUSTRATING THE RELATION BETWEEN GERMINATION OF CONIDIA OF *VENTURIA INAEQUALIS* AND THE TEMPERATURE TO WHICH EXTRACTS OF YOUNG APPLE LEAVES WERE HEATED, BEFORE BEING USED AS CULTURE MEDIA.

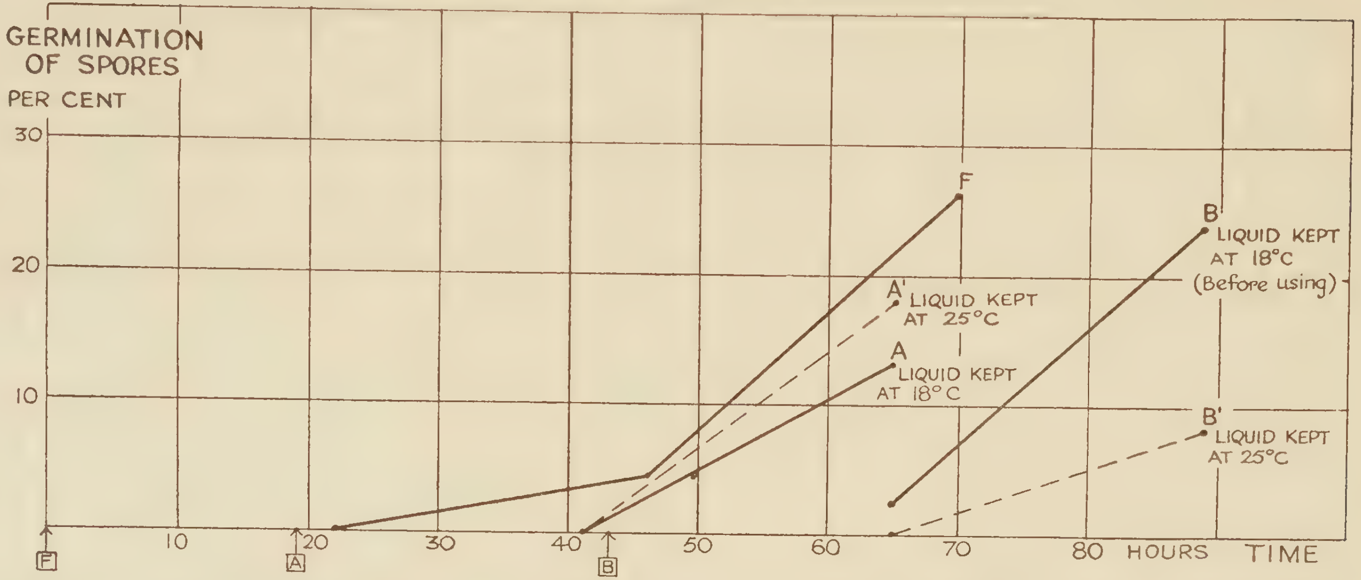


GRAPH III.

GERMINATION OF CONIDIA OF *VENTURIA INAEQUALIS* IN LIQUID EXPRESSED FROM FRUIT OF VARIETY "CAP OF LIBERTY."

DECEMBER, 1928.

INDICATING "STALING" OF THE LIQUID IN THE HANGING DROP.



F, A, B } TIMES AT WHICH HANGING DROPS WERE MADE { FRESH.
19 HRS. OLD LIQUID.
43 ,, ,, ,,

I.E. AGE OF LIQUID WHEN USED AS CULTURE MEDIUM.

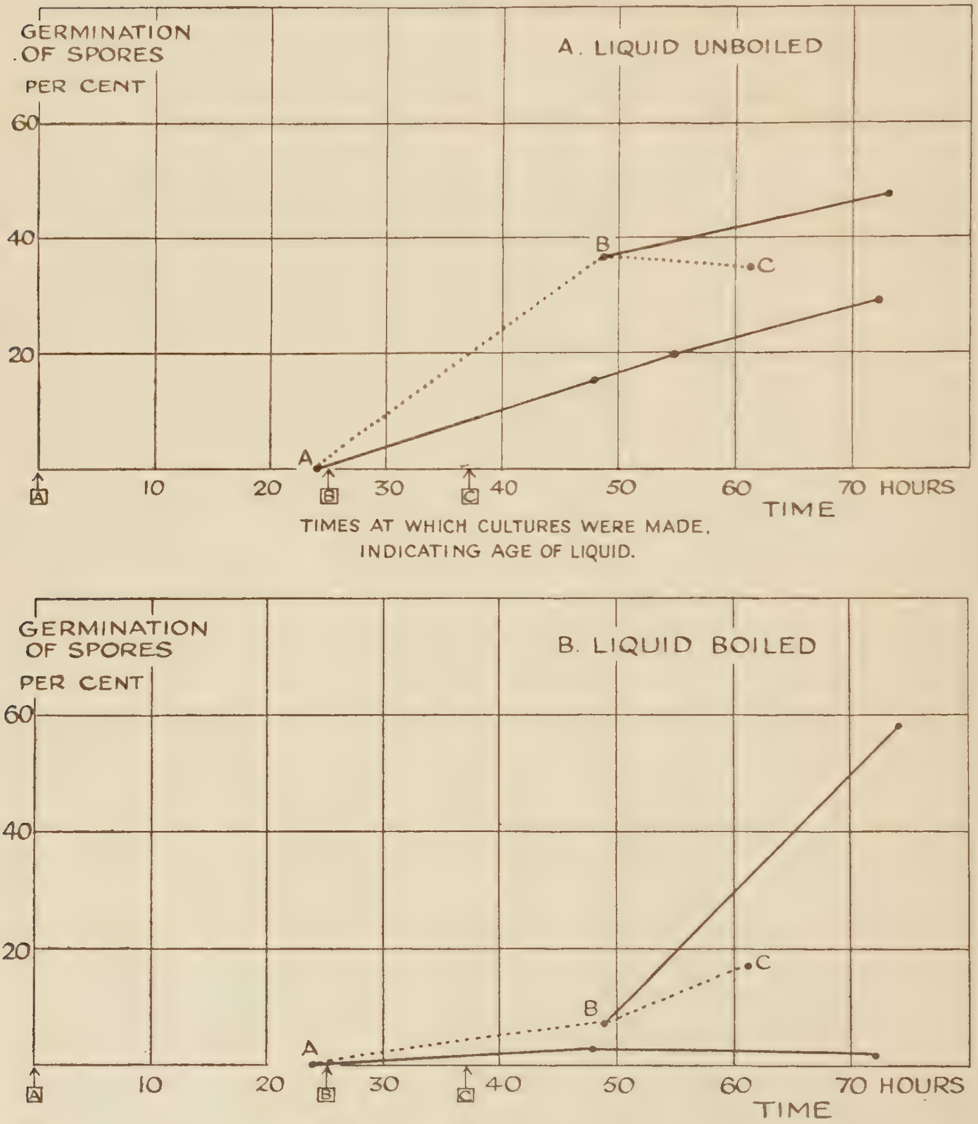
GERMINATION OBSERVED AFTER 22 HRS. AND SUBSEQUENT PERIODS OF 24 HRS.

CURVES REPRESENT LOSS OF TOXICITY IN THE HANGING DROP.

GRAPH IV.

GERMINATION OF SPORES OF *VENTURIA INAEQUALIS* IN LIQUIDS EXPRESSED FROM LEAVES.

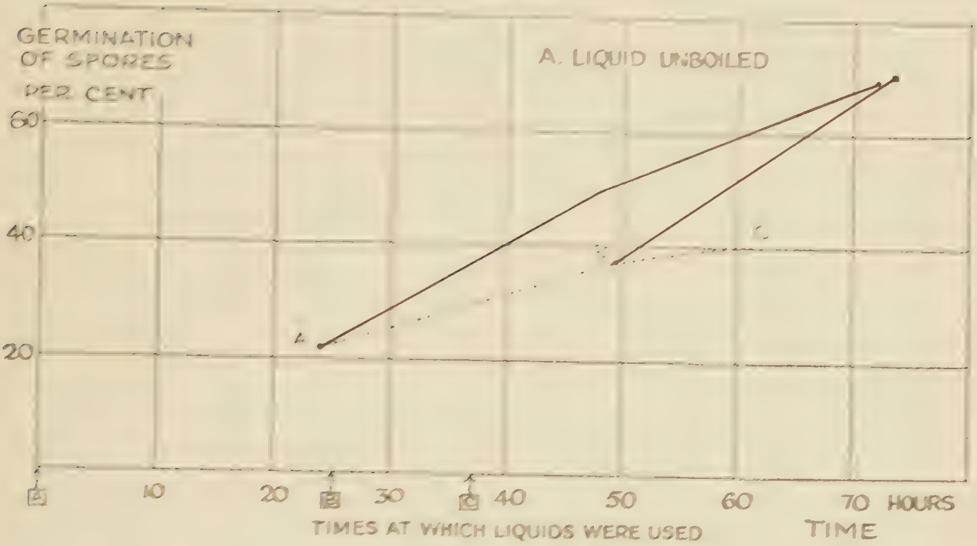
I. VARIETY DABINETT.

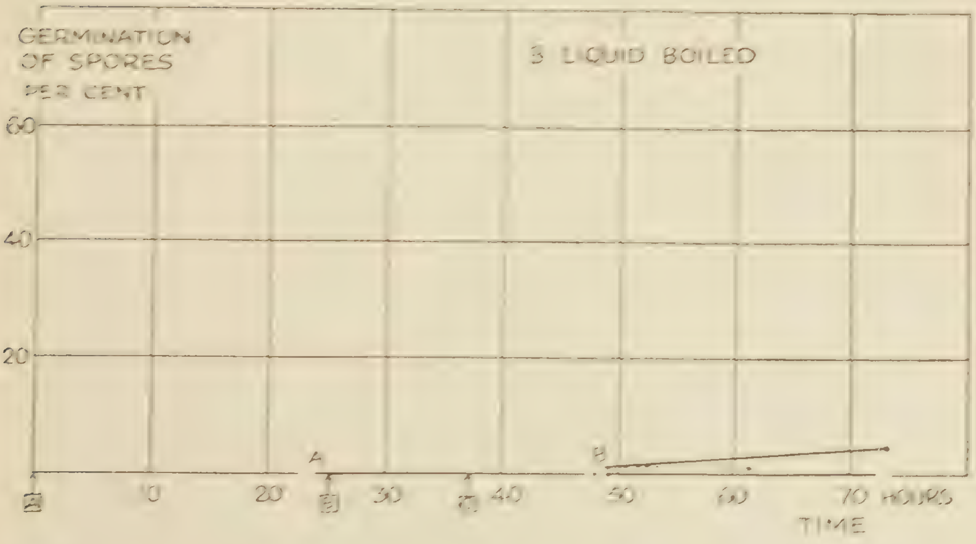


GRAPH V.

II. VARIETY SWEET ALFORD.

[FROM CIDER ORCHARD.]





(Received 6/6/31.)

THE ANTISCORBUTIC VITAMIN OF APPLES. IV.*

By M. B. CRANE and S. S. ZILVA†

FROM

The John Innes Horticultural Institution and the Lister Institute, London.

It has been known for a long time that scurvy occurred when fresh foodstuffs were excluded from the diet for long periods and that the disease could be prevented and rapidly cured when fresh vegetables and fruits were consumed. Systematic experimental work was, however, made possible only by the comparatively recent discovery of Holst and Frölich (1912). These investigators have demonstrated that scurvy, pathologically analogous to human scurvy, could be induced dietetically in the guinea pig and that the disease, as in the case of men, could be prevented and cured by the addition of certain foodstuffs such as fresh vegetables and fruits etc. to the deficient diet.

With the aid of the guinea pig as a test animal a great deal of information has been obtained during the last twenty years concerning the chemical and physical properties of the dietetic principle which prevents and cures scurvy and its distribution in nature. Researches have further revealed that only minute quantities of this unknown substance are required to rectify the dietetic deficiency. It is therefore classed in the category of substances called vitamins and is designated as vitamin C or the antiscorbutic factor.

The main efforts in the study of vitamin C have been directed so far to its bearing on the human and animal organism and to its chemical and physical nature. It is, however, becoming progressively plainer that information on its relation to the plant, in which it is synthesised, would be a valuable aid to the identification of this elusive principle and would further extend our knowledge of its general function in nature. With this end in view one of us has undertaken a systematic study of vitamin C in apples (Bracewell, Hoyle and Zilva, 1930). One of the outstanding results so far obtained is that Bramley's Seedling possesses a markedly higher vitamin C content than the other varieties so far tested. Our cultivated apples are of two fundamentally different kinds, the so-called " diploids " with 34 and " triploids " with 51 chromosomes respectively. Prof. J. B. S. Haldane drew our attention to the fact that Bramley's Seedling is one of the " triploid " varieties in contradistinction to the other varieties tested which are " diploids." Since the " diploid " and " triploid " apples differ widely in various characteristics it was of interest to test a number of other " triploid " varieties in order to ascertain whether a relationship exists between the vitamin C and the chromosome number of the apple. So far we have been able to test only two further "triploid" varieties of authentic origin, namely, Belle de Boskoop and Blenheim Orange. These were obtained for us by Mr. A. C. Painter, of the East Malling Research Station, to whom we are also indebted for the following information:

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* Part 1 appeared in *Medical Research Council Special Report.* Parts 2 and 3 appeared in *Biochemical Journal.* See References.

† Member of the Scientific Staff, Medical Research Council.

Belle de Boskoop.

(*a*) Grown at East Malling Research Station.

(*b*) Soil. Lower Greensand formation (i.e., light loam over ragstone).

(*c*) Manuring for last three years as follows:

1928.	25 cwt.	Shoddy.
	3 ,,	Sulphate of Potash.
1929.	10 ,,	Meat and Bone.
	4 ,,	Sulphate of Potash.
1930.	1 ton	Meat and Bone.
	4 cwt.	Sulphate of Potash.

(*d*) All trees on Malling No. IX. (Jaune de Metz) stock, making small dwarfed trees. Planted December, 1924.

(*e*) Moderate crop—fruit medium size $3\frac{1}{4}''$ to $3\frac{1}{2}''$. Fruit from trees worked on Jaune de Metz stock does not always seem to keep as long as other fruit, at any rate from young trees.

Blenheim.

(*a*) Grown at East Malling Research Station.

(*b*) Soil. Lower Greensand formation (i.e., light loam over ragstone).

(*c*) Manuring for last three years as follows:

1928.	5 cwt.	Meat and Bone.
	3 ,,	Sulphate of Potash.
1929.	2 ,,	Sulphate of Potash.
1930.	5 ,,	Meat and Bone.
	2 ,,	Sulphate of Potash.

(*d*) All trees on Malling No. VII. stock—moderately vigorous, planted March, 1921.

(*e*) Good crop for age. Fruit was between $3''$ and $3\frac{1}{2}''$, i.e., medium size.

As will be seen from the following table, which gives the vitamin C content of a number of varieties,* the activity of both these apples is high. In fact the

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* The cultural details, with the exception of that of the rootstock, of the remaining varieties have already been published elsewhere (cf. Bracewell, Hoyle and Zilva, 1930 and Bracewell, Kidd, West and Zilva, 1931). The possible effects of widely differing rootstocks upon the vitamin C content still remains to be considered.

Dose. gms.	Belle de Boskoop, 1930. East Malling Research Station.	Blenheim, 1930. East Malling Research Station.	Normal Bramley's Seedling, 1928. Burwell.	Lane's Prince Albert, 1929. Exning, near Newmarket.	Normal Cox's Orange Pippin, 1928. Burwell.	Worcester Pearmain, 1928. Burwell.	Newton Wonder, 1929. Eynsford, Kent.	King Edward, 1929. Long Ashton Research Station.
3	+++ - ++++	+	++++	+	+	no protection.	+	no protection.
5	++++	+++	++++	+++	+	no protection.	++	+
10	++++	++++	++++	++++	+++	+	++++	+++
20	++++	++++	++++	++++	++++	+++	vitiated test.	++++

antiscorbutic activity of Belle de Boskoop is equal to that of Bramley's Seedling. On the other hand, it must be noted that Lane's Prince Albert, which is a "diploid," is of the same order of activity as Blenheim Orange. The above evidence, although suggestive of a connection between the high vitamin C content and the high chromosome number in the apple is by no means conclusive. The investigation of a greater number of "triploid" varieties is necessary before definite information can be obtained. This investigation we are endeavouring to carry out.

We are indebted to Miss M. F. Bracewell, B.A., for carrying out the biological tests.

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(*Received* 1/7/31.)

FURTHER STUDIES OF THE BROWN ROT FUNGI.*

VI. BROWN ROT BLOSSOM WILT OF THE MORELLO CHERRY: INFECTION THROUGH UNOPENED FLOWERS.

By H. WORMALD.

East Malling Research Station, Kent.

The Morello cherry, a favourite of the kitchen garden, is often found on commercial fruit farms as bush or half standard fillers interplanted among the standard sweet cherries. In recent years, however, the increasing demand for its fruit for culinary purposes and for bottling has led to its being cultivated more intensively as plantations of bush trees, and with proper attention it has been found to yield profitable crops.

The great disadvantage to its cultivation is that it is very susceptible to infection by *Sclerotinia cinerea,* one of the brown rot fungi. This fungus not only infects flowers, causing blossom wilt, but it extends into the twigs and kills the portions terminal to the infected flowers.

In the acid cherries (varieties of the species *Prunus Cerasus*) the flowers are borne in clusters (*umbels*) at the nodes of the one-year-old twigs (not on spurs as in the sweet cherries), so that when a flower becomes infected the fungus proceeds from the flower directly into the tissues of the twigs, thus interrupting the upward flow of sap, and the leaves and flowers wilt in consequence. In seasons favourable for brown rot infection of the flowers, trees in neglected plantations may have almost the whole of their blossom destroyed in this way and the trees appear as though scorched by fire.

It has been generally assumed that, in blossom wilt, infection takes place through the open flowers and, in fact, inoculation experiments have shown that Morello flowers can be readily infected by placing conidia of *Sclerotinia cinerea* on the stigmas after the flowers have expanded. In 1924, however, the *Monilia* stage of *S. cinerea* was observed on Morello flower buds, suggesting that the fungus is able to infect the flowers before the stigmas are exposed, although the possibility that the flowers might have been killed primarily from some other cause (e.g., late frosts) was not overlooked.

In the spring of 1925, therefore, an attempt was made to induce infection of Morello flower buds by placing on them drops of water containing conidia of a strain of *S. cinerea* which had been obtained from a Morello twig and grown in pure culture. The experiment was carried out in an unheated greenhouse, so that there was no possibility that frost would have any effect on the results.

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* The earlier articles of this series have appeared in the *Annals of Botany* and in the *Transactions of the British Mycological Society*.

Six clusters of unopened flowers were inoculated. The flowers in four of these clusters developed normally. In the other two, however, symptoms of brown rot infection appeared, similar to those observed on trees in the open. On one of these two clusters two flowers became infected. These made no further development after inoculation and were quite dead on the eighth day ; on the thirteenth day they bore many conidial fructifications and the whole twig terminal to the infected flowers was dead. On the other inoculated cluster one flower became infected and bore conidial pustules on the thirteenth day ; the other flowers of this cluster opened normally but then became infected from the base as the fungus entered the spur, and eventually the whole truss of flowers withered.

Although the experiment confirmed the conclusion arrived at from observations in the open, that spores of *Sclerotinia cinerea* reaching unopened Morello flowers could infect and kill those flowers when circumstances were favourable to the fungus, it was considered that such infection was exceptional and that generally the open flowers were the ports of entry for the fungus. In 1930, however, naturally infected unopen Morello flowers were again seen, and from further observations made this year (1931) it would appear that infection of flowers before they open may be of frequent occurrence, particularly if there is a period of wet weather as the buds are bursting.

During the first week of May, when the Morello trees were in full bloom on a stock trial plot on the East Malling Research Station, it was seen that many of the trees bore a number of dead or dying twigs. On many of these twigs most of the flowers had expanded normally and then had suddenly wilted. On a closer examination it was found that in each case there was a cluster of dead, unopened flowers towards the lower end of the dead portion. The fact that these unopened flowers had been arrested in their development and that they bore numerous fructifications of the brown rot fungus was clear evidence that they were the ones which had been primarily infected and that the wilting of the other flowers was a result, not of direct infection, but of desiccation brought about by the fungus invading the twig from the infected flowers. On some infected twigs all the flowers on the affected parts had withered before they had opened (Fig. 3), showing that the fungus had invaded the twig very soon after the infection of the flower buds.

As the primarily infected flowers bore spore-producing fructifications they were obviously a source of infection for the flowers which were then open. All the infected twigs were therefore cut off and burnt.*

The trees were kept under observation and for about a fortnight there was no evidence, from a general examination, that any further infection had occurred. During the last week of May, however, when the flowers had set and the normal fruitlets were about one-third of an inch long, dead and dying twigs were again found on most of the trees. These more recently infected twigs were examined and in nearly every case the flower through which infection had occurred could be detected. As before, the dead portion of a twig was clearly marked from the healthy part below (Fig. 5), and it was found that in one of the flower clusters, towards the base of the infected portion, one flower bore numerous tufts of *Monilia* fructifications on the calyx and along the whole of the flower stalk. This particular flower was, without doubt, the one through which infection had taken place and its appearance indicated that it had opened normally before it became infected. The other flowers of such a cluster bore no pustules at all at this stage, or pustules were present only towards the base of the flower stalk, showing that infection was taking place from the base (Fig. 6).

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* This operation was carried out by Mr. N. H. Grubb to whom the author is indebted for the records of brown rot infections on the Morello plots.

Many infected twigs were examined but in no case was there evidence that on any one twig the infection had originated in more than one flower. Inoculation experiments carried out in the greenhouse under controlled conditions have also shown that the inoculation of a single flower is sufficient to cause the death of that part of the twig terminal to the node on which that flower is inserted. Thus the infection of a single flower may cause the death of a length of twig several inches long (up to a foot or fifteen inches in cases where the infected flower is situated towards the lower end of a long twig) and bearing many flowers.

It is evident, therefore, that in Morello cherries there may be two waves of infection, one through the unopened flowers, the effect being noticed about the time the tree is in full bloom, the other through the open flowers, the result in this instance showing after the fruit is set. In each case there is an interval of about a fortnight after infection is initiated before the effect becomes conspicuous by the wilting of the flowers and leaves on infected twigs ; during this period the spores germinate, the germ tubes penetrate the flower, and the fungal hyphæ grow through the tissues of the flowers down the stalk and into the twig. The flowers primarily infected are of course killed first, but such individual dead flowers remain inconspicuous among a mass of blossom and the result of the infection remains unnoticed until the twigs are invaded with the consequent wilting of their flowers and leaves.

The fungus passes the summer and autumn within the dead twigs and the remnants of flowers and leaves which tend to persist on such twigs. During the following winter and spring grey cushion-like bodies about one-twentieth of an inch in diameter appear on the surface (Fig. 2). These are the *Monilia* fructifications, very similar to those which arise on newly infected flowers ; they are dense tufts of branched chains of conidia. The conidia are very small (only about one two-thousandth of an inch in length) and as they are very light and powdery they are easily carried about by the wind. These fructifications on the dead twigs produce the spores which start new infections in spring as the tree comes into flower.

It follows that, in order to prevent further infection of the flowers, the twigs killed one year should not be allowed to remain on the trees until the following spring. It is advisable to cut them out as soon as the wilting is observed, for then their withered flowers and leaves render them so conspicuous that they can hardly be overlooked. Later in summer, as the trees come into full leaf, the dead twigs may become partially hidden by the foliage. Again since the flowers infected early (before opening) bear fructifications, with numerous spores, about the time the trees are in full bloom, such flowers may serve as sources of infection for the blossoms which are open, so giving rise to the second wave of infection, which, as shown above, originates at the time the trees are in flower. The early removal of the twigs bearing flowers killed during the first wave of infection will therefore reduce the chances of infection of the open flowers. If, for want of labour, the twigs cannot be cut out during summer the operation may be postponed until the winter, but careful search is then necessary in order to ensure their complete removal.

These precautions are necessary even in years when there is little infection, for if the weather during the spring of the following year is favourable for the brown rot fungus the fructifications on a few dead twigs may produce sufficient spores to cause a serious outbreak of blossom wilt. In 1924 and again in 1931 (years of severe infection following, in each case, a year when infection was comparatively slight) there was much damage caused in Morello plantations where the few infected twigs of the previous year had been passed over as negligible.

Experiments carried out by Salmon (1) in 1906 show that brown rot infection of Morello cherries can be greatly reduced by spraying the trees with Bordeaux mixture just before the flowers open and again just after the blossom has fallen. As the primary object of spraying Morellos is to protect the flowers, and since, as shown in this article, infection can take place through the unopened flowers and also through the open flowers, it would appear that the period for effective spraying is shortly before the flowers expand. The blossom wilt fungus may also infect the young cherries, causing fruit rot, but the loss on this account is usually negligible and it is probable that the post-blossom application of the spray is not nearly so important as the pre-blossom application.

In addition to the careful cutting out of all infected twigs it is advisable therefore (especially for trees which have shown heavy infection in the previous season) to spray the trees with Bordeaux mixture as the flower buds appear, in order to minimize the chances of early infection by spores carried in the wind from dead twigs which may have been overlooked, or from infected trees in the neighbourhood. In this connection it may be remarked that some measure of control of the brown rot infection of the flowers of apricots in France has been obtained by spraying the trees before the flowers open with Bordeaux mixture (2).

Although infection of the fruit by *Sclerotinia cinerea* is rarely, if ever, severe, it should be borne in mind that infected cherries are a source of danger, and no mummified fruit should be allowed to remain on the trees, for they, like the dead twigs, produce fungal fructifications during the winter and spring and so serve to infect the new blossoms.

The fungus responsible for the blossom wilt disease of Morello cherries is referred to here as *Sclerotinia cinerea.* This species is known to include two forms, *S. cinerea* forma *pruni* and *S. cinerea* f. *mali,* the former infecting plum and cherry trees, the latter causing a blossom wilt of apple trees. In pure culture the Morello fungus is found to conform to *S. cinerea* f. *pruni* rather than to f. *mali.* Experiments are on hand to obtain confirmatory evidence of its identity with the form infecting plums and cherries, and so far as they have gone these experiments have supported the view that blossom wilt in Morellos, sweet cherries and plums is caused by the same fungus. It has been found that *S. cinerea* isolated from plum and cherry can readily infect Morello flowers, producing typical blossom wilt and killing the twigs. Inoculations on plum and cherry flowers with *S. cinerea* isolated from the Morello have up to the present given inconclusive results, but this is believed to be due to the somewhat unfavourable conditions under which the experiments were carried out. The flowers actually inoculated showed the early symptoms of infection and were killed, but infection did not extend into the spurs. As, however, similar results were obtained with strains of the fungus isolated from plum and sweet cherry trees they may be considered as confirming, rather than disproving, the supposition that blossom wilt of the Morello, sweet cherries and plums is caused by one and the same fungus, viz., *Sclerotinia cinerea* forma *pruni.*

Fruit rot of Morello cherries is also produced by the closely related fungus *Sclerotinia fructigena,* which is the cause of the common brown rot of apples and pears. On Morellos it infects the ripening fruit but the damage is usually not serious enough for special precautions to be taken against it.

Another disease of Morello trees and one which may be confused with blossom wilt infection is caused by a bacterium which is closely related to (and perhaps identical with) one which is responsible for bacterial canker in plum trees and sweet cherries. In Morellos it infects chiefly the young branches, checking their development or killing them back. As in blossom wilt, the affected parts should be cut away below the infected region, as soon as the disease is noticed.

Bearing in mind the extreme susceptibility to brown rot infection shown by the Morello cherry, as described above, involving careful cutting out of diseased

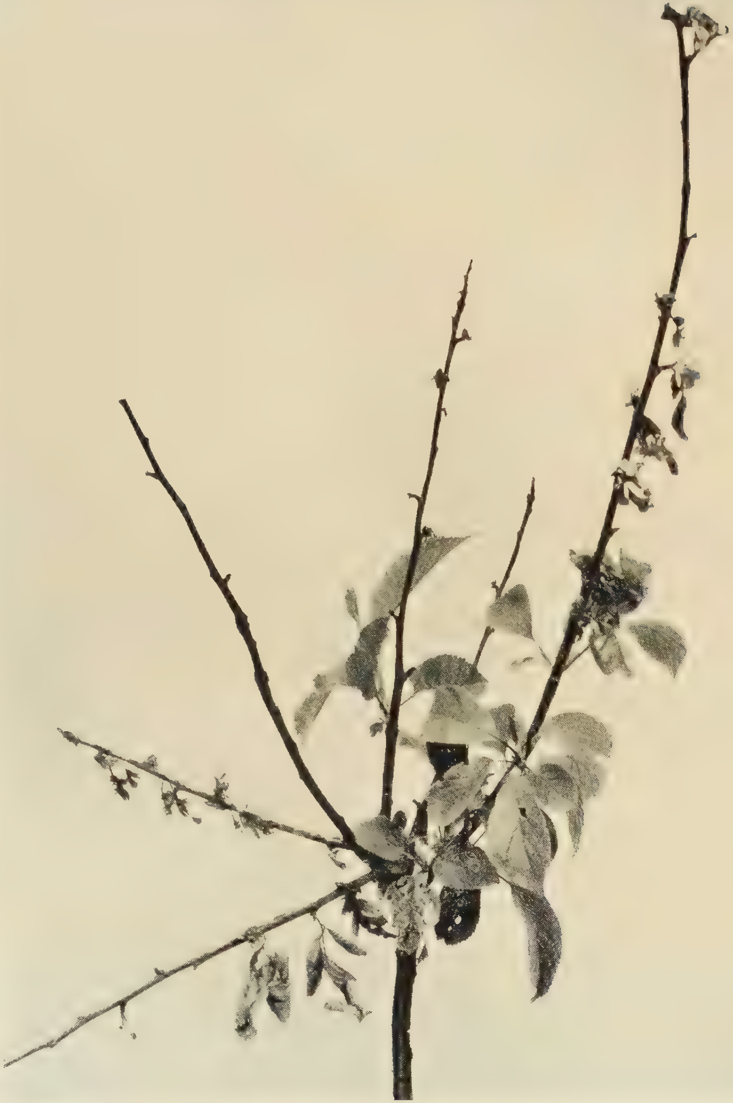

FIG. 1.

Brown rot infection of Morello Cherry. Three twigs killed the previous year and three during the current season.

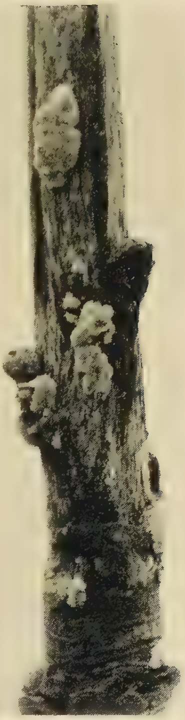

FIG. 2.

Portion of a dead twig enlarged, showing the conidial fructifications of *Sclerotinia cinerea*. ×5.

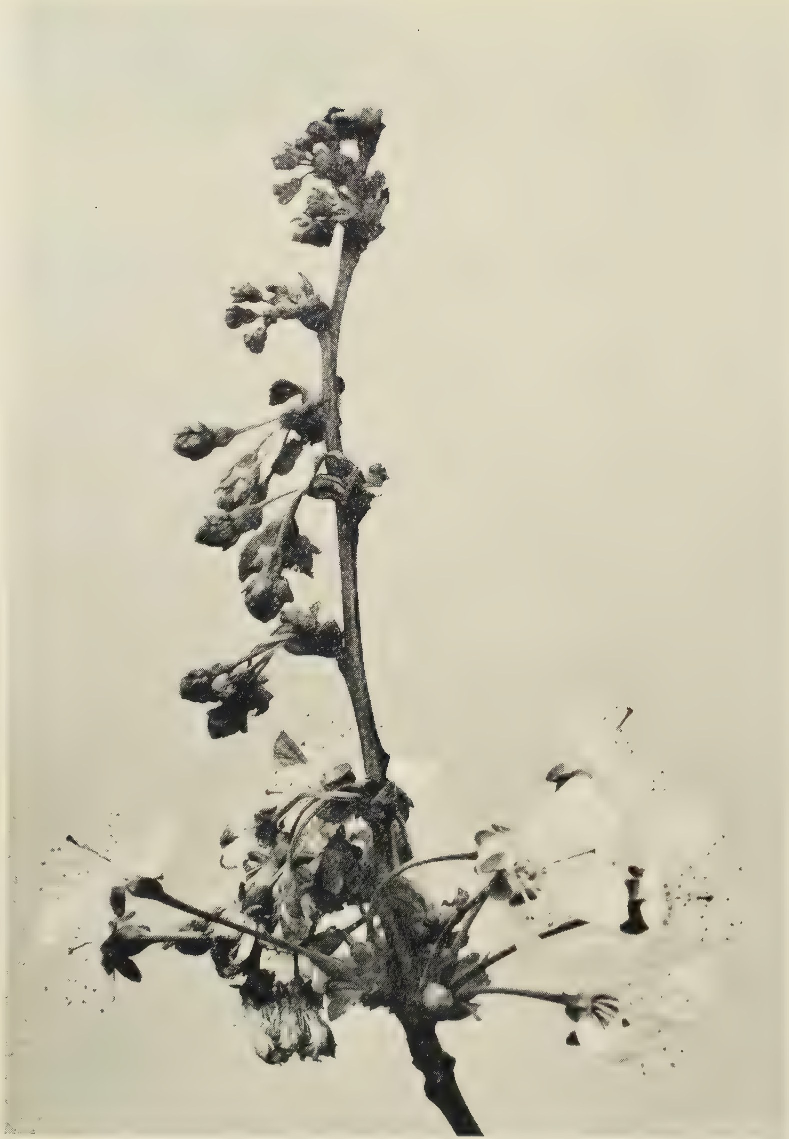

FIG. 3.
Result of infection through unopened flowers.

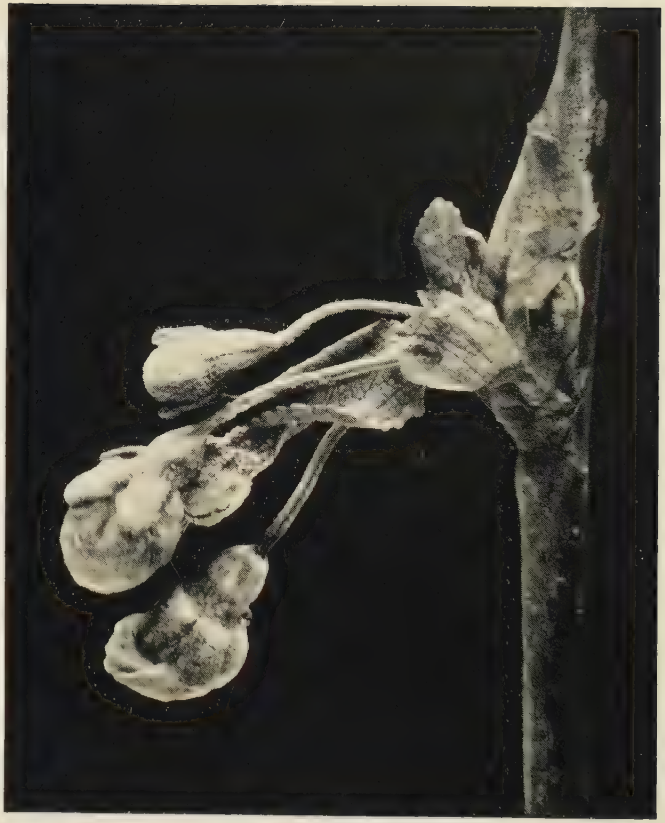

FIG. 4.
An infected cluster of unopened flowers, enlarged, to show the fructifications of the fungus on the flowers and flower-stalks.
× 2½.

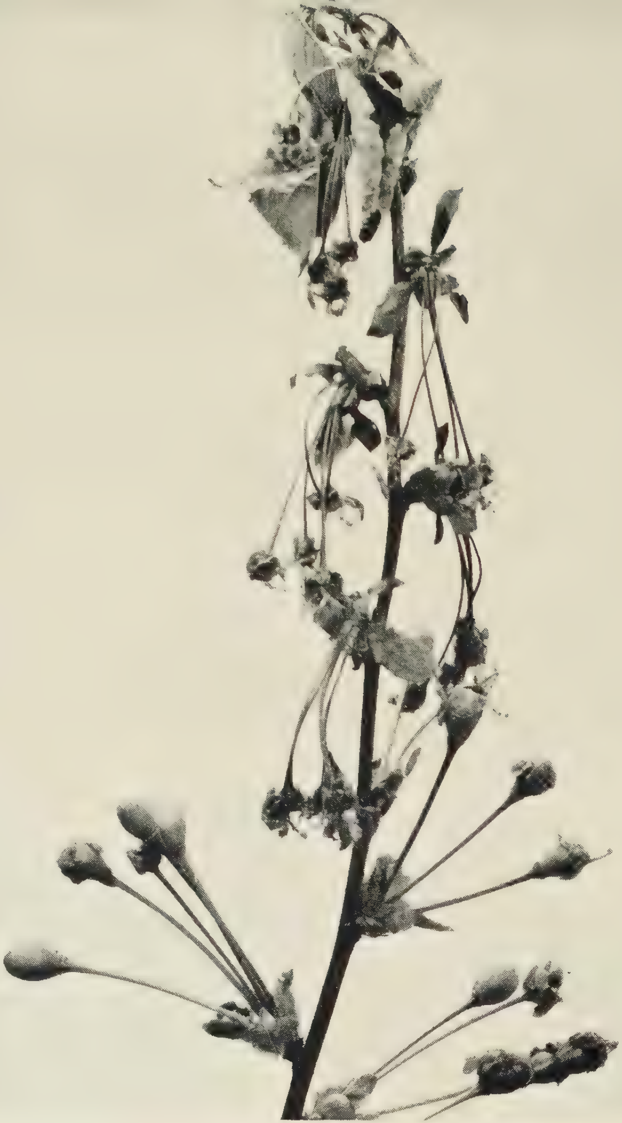

FIG. 5.

Result of infection through open flowers.

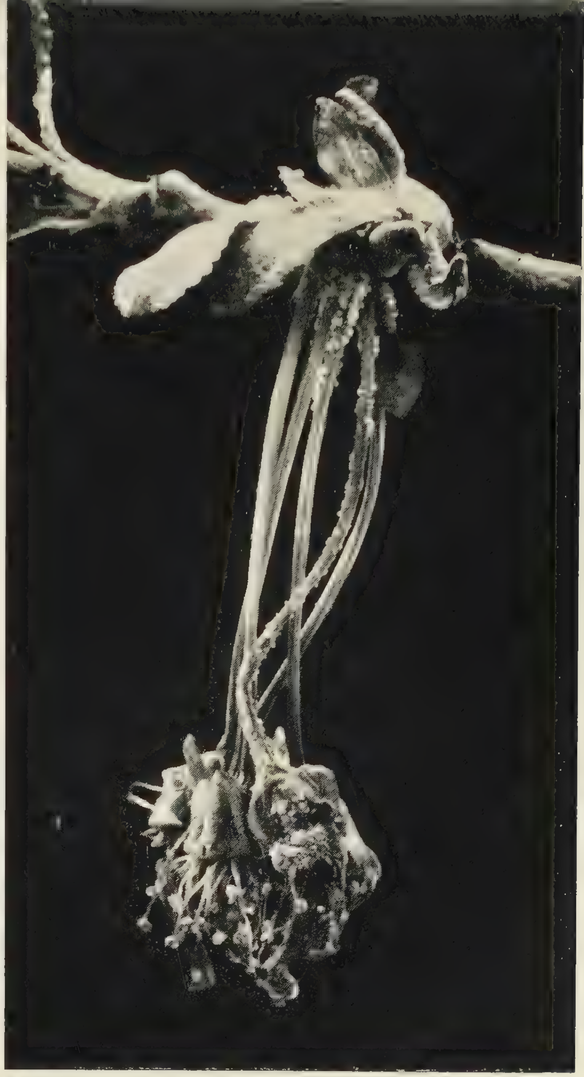

FIG. 6.

A cluster of flowers which had opened before they became infected. The flower through which the fungus entered shows numerous fungal fructifications on its stalk; the other flowers are being infected from the end where they join the twig. ×2½.

twigs if the trees are to yield good crops, it is evident that this variety of cherry should be grown as bush trees, for it is only on such trees that the control measures advocated are likely to be carried out with any hope of success. The condition of standard and half standard Morellos sometimes seen interplanted among sweet cherries is often deplorable.

SUMMARY.

The Morello cherry is very susceptible to infection by the fungus *Sclerotinia cinerea* forma *pruni*, which infects the flowers, causing a blossom wilt ; it then extends into the twigs and kills them back for several inches.

Two waves of infection have been observed. The first occurs initially before the flowers open and the result becomes noticeable, by the withering of infected twigs, about the time the trees are in full bloom. The second occurs when the trees are in flower, and the twigs wilt about a fortnight later.

Diseased twigs if left on the trees become sources of infection the following year, so they should be removed as early as possible. Spraying with Bordeaux mixture shortly before the flowers open is also recommended.

For these control measures to be carried out effectively Morello cherries should be grown as bush trees.

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BOOK REVIEWS.

THE JOURNAL OF THE SOUTH-EASTERN AGRICULTURAL COLLEGE, WYE. No. 28, 1931.

The present number of the Wye College Journal is well up to the high standard set by its predecessors, and the various articles give a good indication of the wide range of the investigations carried out at the College. In addition to the general reports of the activities of the various departments of the College, there are, as usual, a number of individual contributions, of which the following will be of particular interest to readers of this Journal.

Further Experiments on the Control of Apple Scab, in which the efficiency of lime sulphur and Bordeaux Mixture was tested on various varieties of apple are described by N. B. Bagenal, W. Goodwin, E. S. Salmon and W. M. Ware. Methods of emulsifying oils for use as spray fluids are discussed in an important paper by H. Martin. Oleic acid, used in conjunction with a dilute solution of caustic soda, was found to give satisfactory emulsions both of vegetable and mineral oils, including tar distillates and petroleum oils, the employment of proprietary emulsifiers thus being rendered unnecessary. Entomology is represented by papers by H. F. Barnes, who contributes a supplement to his previous account of the Gall Midges affecting fruit trees; by S. G. Jary, who gives observations on the Strawberry Bud Weevil, and on the Winter Moth attack on Fruit Trees in Berkshire in 1929-1930; and by M. D. Austin, who describes the biology of the Apple Capsid and the Common Green Capsid bugs.

In view of the increasing importance of soil classification and survey work in agriculture, the article by Prof. L. L. Lee, of New Jersey, U.S.A., is of especial interest. It deals with the possibilities of an International system of soil classification and gives detailed accounts of the various methods at present in use, together with a comparative study of South-East England and Central New Jersey. A.S.B.

HOW TO LIVE IN ENGLAND ON A PENSION. By "MAUSER." (W. Thacker & Co., London.)

This little book has been the subject of many enquiries. Rumour, that lying jade, had it that here was a man who had spent the best part of his life soldiering in the more uncomfortable parts of the Empire, retiring on a meagre pittance grudgingly handed out to him by an ungrateful country, and behold him in three years well on the way to making a fortune. And how? By growing apples!

It is not, however, quite like that. The title of the book is alluring but perhaps insufficiently definite. The pittance is £800 a year, supported by a capital of £2,000. One is tempted to ask, "Why bother with the apples?" Not so the author. No car for him on this starvation income, but a second-hand push-bike while his lady wife must walk! As he remarks in substance, those who walk most live longest. Here is not the England of his dreams and simply and forcefully he points out its failings.

Apart from the enjoyment which the mere reading of it brings it is packed with sound information. Every question that is likely to interest an intending "settler" is thoroughly examined. Separate chapters are allotted to choice of locality, the purchase of a house, furnishing, education and finance, all discussed in a manner thoroughly practical but never dull. And now having got his family comfortably installed the settler looks around for something to occupy his spare time and augment his pittance. His choice falls on apples. Throughout the settling process his motto has been "nothing but the best" (which possibly has some bearing on the too facile evaporation of the annual eight hundred), and he proposes to apply this principle to apple growing.

Briefly the plan is to grow Cox's Orange Pippins de luxe on cordons and to sell them, packed in fancy trays, to the north-midland markets, where the author supposes good fruit is scarce and the inhabitants have the will and the money to buy it when it appears. No attempt is made to give instruction as to cultivation or choice of ground, it being sensibly suggested that the necessary advice should be obtained from the local Research Station or some other competent body. The area suggested for the orchard is two acres, costing £100, to be planted with 6,720 Cordon Cox on Jaune de Metz stock at a cost of £700 for the trees only. Labour will consist of the settler himself and one assistant, and this with sundry expenses brings the first year's outlay up to £978. The second year's expenditure is £40, which is fortunately exactly balanced by the sale of apples and from the fourth year onwards the receipts for fruit amount to £500 annually, which after deduction of expenses leaves a profit of never less than £360. The author claims to be in the third year of practical experience and his estimate is based on figures supplied by a firm of leading fruit growers and nurserymen (whom he names). His profit for the third year is scheduled at £240. One sincerely hopes he secures it.

BACTERIAL DISEASES OF STONE FRUIT TREES IN BRITAIN.

III.* THE SYMPTOMS OF BACTERIAL CANKER IN PLUM TREES.

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INTRODUCTION.

FRUITGROWERS in this country have experienced great losses in their plum plantations owing to the death year by year of a number of trees. The trouble is not uncommon in the nurseries, but when it appears in plantations the loss is of far greater economic importance, particularly when trees just coming into full bearing are attacked, for they are usually killed outright in the course of a season and have to be replaced by others from the nursery.

Various possible explanations of this so-called "die-back" have been put forward from time to time, but generally no experimental evidence of a conclusive nature has been presented in confirmation of the conclusions drawn.

A disease of plum trees which appears to be identical with the one which is the subject of this paper was described in 1902 by G. Massee (10), who attributed the disease to infection by the fungus *Eutypella Prunastri* which has *Cytospora Prunorum* as its conidial stage.

In 1907 Aderhold and Ruhland (1) described a bacterial disease of cherry trees in Germany and incidentally referred to a similar disease of plum trees which they thought was also caused by bacteria ; the plum disease, however, was not described in detail, and was not investigated further.

A die-back of branches of Greengage and Victoria plum trees in the neighbourhood of Cambridge was attributed by Dowson (8), in 1913, to *Dermatella Prunastri*, although the only result he got from his inoculations with that fungus was that, "A slight discoloration was observed in the immediate neighbourhood of the wood. A fair amount of gumming had taken place and hyphæ similar to those in nature were present in the wood."†

Belgrave (4) found species of *Cytospora* frequently present on dead or dying plum trees and in a paper published in 1915 concluded that *Cytospora leucostoma* (= *Valsa leucostoma*) was the probable cause of the trouble, although his inoculation experiments had all given negative results.

In 1920 Wiltshire (11) gave a brief account of organisms present on a number of plum trees showing die-back symptoms. He found fungal hyphæ in the cortex and wood, associated with *Cytospora* fructifications, but he goes on to state, " In many sections of the diseased cortex, however, certain gelatinous masses, with numerous small, rod-like bodies embedded in them, were also found, which strongly resembled small encapsuled bacteria.

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* For Parts I and II of this series see References 12 and 14.

† Mr. F. T. Brooks, who is familiar with the disease described by Dowson, informs me that he considers the *Dermatella* die-back to be distinct from the bacterial disease.

" . . . There appears to be no doubt that the occurrence of these suspicious looking bodies is fairly general in trees of various varieties affected with die-back." On taking over another post Wiltshire was unable to continue this work.

Miss Cayley (7) in 1923 described *Diaporthe perniciosa* as a fungus very frequently found on plum trees showing die-back and by inoculations with that fungus was able to induce wilting of young green shoots of peach and nectarine ; on two-year-old plum twigs, however, the only effect of the inoculations was " considerable disturbance accompanied by gumming," and the typical symptoms of die-back were not produced.

In the same year that Miss Cayley's paper appeared, Mme. Arnaud (3) described a disease of plum trees in France with symptoms resembling those of the disease as observed in England. The two fungi found associated with the disease in France were *Valsa leucostoma* and *Eutypella Prunastri*. That author concluded that the fungi were not serious parasites but caused loss when the trees were growing under unfavourable conditions ; there was no mention of inoculation experiments to test the parasitism of the fungi however.

Briton-Jones (5) in 1925, reviewed the die-back problem up to that date and considered that there was insufficient evidence to prove that the disease was caused by either fungi or bacteria. He concluded that *Diaporthe perniciosa* and, in some cases, *Cytospora* spp. were weak parasites, only attacking trees which had been enfeebled by adverse cultural conditions. He found bacteria in the lesions on diseased trees but stated that " Attempts to grow the bacteria in pure culture on artificial media have failed." His conclusion was that " there is strong evidence that none of these organisms is the primary cause of the trouble " and that the factors chiefly concerned in producing die-back are excessive soil moisture and insufficient soil moisture.

Marsh and Nattrass (9) continued the work on *Diaporthe perniciosa* and found that it could cause lesions on plum trees ; in no case was a stem killed by the inoculations, but they describe one canker which was 6 cm. long by 2 cm. broad.

It will be seen from the above review of the papers on the plum die-back problem that the trouble has been generally attributed to various fungi that have been encountered from time to time on affected trees. Some of these fungi appear to be weakly parasitic but no one has succeeded in reproducing the typical symptoms of the disease from inoculations into woody stems or branches.

During July and August, in 1924 and 1925, the present writer examined many die-back trees from the plum trial plots on the East Malling Research Station. Various fungi were present on the affected trees and bacteria were invariably found, often in dense masses particularly in the tissues bordering on the healthy parts. It was inconceivable that the trouble in this case was due to unsuitable water relations in the soil, for the trees made excellent growth (no stunting of the shoots such as would have occurred if the trees were growing in unsuitable soil) until the lesions appeared on the stems, and other trees growing in close proximity to affected trees continued to develop vigorously. It was believed therefore that the damage was brought about by a virulent parasite and, from the presence of the bacteria at the borders of the lesions, that these bacteria were the primary cause.

Experiments had already shown that a bacterium was responsible for a wilt disease of young green shoots of plum trees and it was suspected that the same organism produced the lesions on stems and branches. Attempts to isolate and cultivate the bacteria present in the stem lesions failed at that time (July and August). It was argued, however, that the bacteria must have been alive earlier in the year, so in 1926 a careful examination of trees on a trial plot on the Station was made during the last week of April, as the trees were coming into leaf. From their general appearance all appeared healthy, but, on certain trees, holes (caused by the Shot Hole Borer beetle, *Anisandrus dispar* Fab.) were detected in the bark. On cutting into the bark of such trees the cortex was found to be discoloured for a considerable distance along the stem. On a microscopical examination of tissues near the borders of the lesions bacterial masses were found and isolation plates prepared from such material yielded an organism which came up readily and could be cultivated without difficulty. During the following month (May), similar trees and others without beetle holes showed depressed areas of bark corresponding to lesions with discoloured tissues, and other isolations, of what appeared to be the same organism, were obtained from a number of affected trees. Later, however, in July and August, as the lesions became more pronounced and the foliage of diseased trees showed distinct symptoms of ill-health, although bacteria could be found in the lesions they usually refused to grow in culture, so that it was apparent that the bacteria in the cankers die out during the summer, and this fact explains the difficulty, or entire failure, that previous workers experienced in attempts to cultivate them.

This form of die-back in plum trees has now been under investigation for some seven years at the East Malling Research Station and repeated experiments have shown that the typical symptoms of the disease with cankers and finally the death of the parts above the lesions can be reproduced by inoculations with pure cultures of the bacterium, which can be isolated not only from infected stems but also from lesions on young shoots, leaves and fruit. As the death of a tree is the result of a bacterial lesion which causes a canker-like wound on the stem, this type of die-back is here referred to as " Bacterial Canker." This term was applied to the disease in brief preliminary descriptions that have already appeared (12, 13).

Field observations have been made on trees of various ages, from maiden trees in nursery rows to those in established fruit-bearing plantations producing good crops.

These observations have been made mostly at East Malling, but they have been supplemented by others made on various Kentish fruit farms and by an examination of specimens received from other counties.

THE SYMPTOMS OF BACTERIAL CANKER IN ESTABLISHED PLANTATIONS.

In typical cases of bacterial canker in the plantation, trees make good growth for one or more years and then, during the course of a single season after becoming infected, they produce unhealthy-looking foliage in the spring and die during the summer. The first noticeable sign of disease on such trees is the pale-green or yellowish colour of its foliage which may be observed soon after the leaves unfold. At first this divergence from the normal is hardly noticeable and often can only be detected by carefully comparing one tree with another and noting any which appear to have slightly yellowish leaves. Within a few weeks, however, the difference in colour becomes more pronounced and generally from the middle of May onwards diseased trees are easily recognized by their yellowish foliage. The yellowing is more conspicuous in some varieties of plums than in others. A variety that shows it distinctly is Czar.

The difference in leaf colour is usually accompanied by a check in the development of the young shoots, those developing from terminal buds of the branches often remaining very short. Such shoots may be no longer than one or two inches when corresponding shoots on normal trees are a foot long or more. This, however, is not a constant character and some infected trees produce well-developed shoots.

Another leaf character of infected trees is an upward curling of the margins of the leaves, which thus appear more or less " rolled." This again is shown more clearly in some varieties than in others. On Czar it is not so noticeable as on Victoria. On Bradley's King, a variety of damson, it is a conspicuous feature of infected trees. The leaves, apart from their being rolled, are generally slightly narrower than normal leaves. The whole effect of fewer, narrow, rolled leaves, often gives a cankered tree the appearance of having " thin " foliage, particularly in certain varieties. Thus while cankered Czars are conspicuous by reason of their yellow leaves, in Victorias the " thin " foliage is a more characteristic feature.

In most cases the foliage of trees showing these initial symptoms wilts and finally withers, and the whole tree dies, except the lower part of the stem and the roots. Some trees wither when the fruit has grown nearly to its full size. Others may retain unwithered foliage until autumn, but if they are girdled by cankers they fail to come into leaf the following season.

These characters as shown by trees with cankers on the main stem, are also seen on individual branches when the cankers are confined to the branches themselves ; thus some trees bear normal foliage except on those branches actually cankered. Sometimes a canker at the crotch is confined to one side of a tree and to the base of the branches arising on that side ; in such cases one side of the tree bears normal foliage while the other side shows the symptoms described. When individual branches only are killed the rest of the tree remains healthy during that and succeeding years unless new infections occur, for, as mentioned below for stem cankers, the damage caused by any one canker is confined to one season, for these bacterial cankers are not perennial.

Early in spring, when the leaves are beginning to unfold, before the yellowing can be observed, it is usually impossible to distinguish infected trees from normal ones by a superficial examination, for the stem lesions at that time are not noticeable. It is only when the stem begins to increase in thickness that the infected parts can be seen as sunken areas. As the cambium is destroyed in these areas no new wood or bark develops there, and the demarcation of the lesions becomes conspicuous only when the cambium of the healthy tissues around lesions resumes its function. During the summer the borders of a lesion are usually well defined, for by that time the canker has ceased to increase in size and where it has not girdled the stem the tissues bordering the lesion produce callus. This eventually causes cracks in the bark and a definite canker is seen.

When the borders of a canker are observable by reason of the ridging caused by the callus, a cut made across the ridge, passing down obliquely through the bark to the wood, shows a sharp demarcation of the healthy and the infected tissues, the dark colour of the latter forming a strong contrast with the almost white normal tissues of the bark. In the early spring a cut made along any irregularities in the bark, especially ridges suspected of indicating canker, will show clearly whether the stem is infected in that region.

The stem cankers are usually from one to three feet in length and the longer ones therefore extend almost the whole length of the stem, from the ground to the level where the primary branches take their origin (the " crotch "). A canker may arise in any part of the stem. It usually occupies an area near or around the middle of the stem, but it may appear at the crotch so that the upper part of the stem and the lower portions of one or more branches are infected, or it may be at the base of the stem extending from the ground level upwards for a foot or so. Only rarely have two cankers been found on one stem.

Most of these observations have been made on trees grafted or budded "low," i.e., with the union at or near ground level. The cankers rarely extend below the level of the ground, so that the stock, and therefore the roots also, remain sound. This is a feature shown by most trees affected with bacterial canker and serves to distinguish this disease from die-back caused by unsuitable soil conditions which would result in decay of the roots or would check their development, or from disease caused by root-rotting fungi as *Armillaria mellea* or *Rosellinia necatrix*.

In many instances, therefore, the lower portion of the stem is not killed, and as the roots remain healthy and are still functioning, the death of the upper part of the tree causes dormant buds, situated on the lower part of the scion stem, or on the stock, to be stimulated into development and they grow out into strong leafy shoots. The colour of the foliage on such shoots is the normal green and so contrasts with the pale yellowish leaves borne on the parts above the canker.

The extension of a canker is usually far greater in the vertical direction than horizontally. It thus sometimes happens that even a long canker may not girdle the stem. A striking example of this was seen on a Warwickshire Drooper plum tree growing on a plot at East Malling, and noticed as showing disease symptoms in the spring of 1928. This tree was five years old and its stem was about two and a half inches in diameter. During the first week of June of that year its leaves were somewhat rolled and yellowish as compared with neighbouring trees of the same variety, and its shoots were shorter than normal, the longest measuring about six inches. A slightly sunken area could be detected extending along nearly the whole length of the stem. From the upper end of this canker a small piece of bark was removed ; this was found to contain numerous bacteria and an organism was isolated resembling that which had been isolated from other cankered trees. The tree, because of its general unhealthy appearance and the presence of bacteria in the stem, was therefore marked down as one which was expected to die during the course of the summer. Towards the end of July the leaves were still slightly paler than normal. The cankered area was by that time clearly indicated and it was seen to extend from nine inches below the crotch to four inches above ground level—a distance of thirty-five inches. The canker, along the greater part of its length, approximately half girdled the stem, but near the upper end the strip of healthy bark was only one inch wide or less than one-sixth of the whole circumference. During September the foliage was obviously paler than normal and this was accompanied by premature leaf-fall. The curling upwards of the edges of the leaves was very noticeable at this time. The longest shoots were still only about six inches long (they had therefore made no appreciable growth since the first week of June), while on corresponding healthy trees terminal shoots were up to twenty inches long. During 1929 the cankered area did not increase in size (it was still thirty-five inches long), but it became very conspicuous by reason of the development of callus at its edges. The tree had an almost normal appearance, though when compared with other trees its leaves were seen to be slightly pale and rolled, while its shoots were short. The canker at its upper end faced south and at the lower end north-east ; it thus formed a loose spiral passing downwards from right to left.* The tree survived through the season of 1929, but during a gale in the spring of 1930 the stem was broken across at its weakest place, that is, where the canker had nearly girdled it. (See Fig. 9.)

During the summer months fungal fructifications are almost invariably to be found on the cankers. The forms mostly met with are species of *Valsa* and *Cytospora*, and the *Phomopsis* stage of *Diaporthe perniciosa*. The presence of these fungi on the cankered areas, while the trees are still alive, suggests that they are parasites, but this conclusion is discountenanced by the fact that other fungi are also often present. Thus during July and August, 1924, many trees showing the symptoms of bacterial canker were examined and at least nine different forms of fungal fructifications were found, including, besides those already mentioned, *Pestalozzia Hartigii* and the conidial stage of *Dermatella Prunastri*, together with immature forms which were not identified.

If the diseased tissues are examined in spring or early summer, however, the only organisms present in the tissues towards the upper and the lower extremities of the canker are bacteria, and these are in dense masses. Later in summer the bacterial masses may still be found but, as already mentioned, when culture plates are prepared from them either there is no growth at all or colonies appear which are different from those of the organism which has been found to cause the cankers. The fact that the dying out of the bacteria corresponds with the cessation of the development of the cankers and that at this stage the fungi mentioned are still flourishing on the lesions, is further evidence that the bacteria are the primary cause of the death of the tissues and that the fungi grow only on the parts already killed by the bacteria.

In an inoculation experiment carried out on young plum trees in the autumn, using a pure culture of the bacterium, various fungi appeared on the resulting cankers (caused by the bacterium, for control trees remained healthy) during the course of the following summer. These results suggest that the bacterium destroys the tissues in winter and spring, and this conclusion is borne out by inoculations made monthly throughout the year, when it was found that October and November were the months in which the inoculations were most successful, resulting in well-defined cankers, while wounds inoculated during the summer months healed normally and without cankering.†

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* This spiral form has been observed in other long cankers and probably bears some relation to the phyllotaxis of the primary stem.

† See p. 250.

As stated above, infections on established trees usually result in their death during the course of the summer. When such trees fail altogether to come into leaf in the spring it is an indication that they had shown the symptoms of disease the previous year but that they had retained their foliage until the end of the season and so had not been removed.

The disintegration of the cortical tissues is accompanied by the production of water-soluble gum, but in plum trees this gum is usually not sufficiently abundant to flow out to the exterior. In one variety, however, the damson Bradley's King (mentioned below as being very susceptible to the disease), stem cankers often show an exudation of gum, and in this variety one of the first obvious symptoms of the disease in the early spring is the appearance of gum on the surface of the stem.

LOSSES CAUSED BY BACTERIAL CANKER IN PLANTATIONS.

The extent of the losses that may sometimes be caused by this disease in plum plantations may be estimated from certain cases which have come under the notice of the author. A fruit-grower at Marden, Kent, sent to East Malling in June, 1928, a stem of a Czar plum tree showing bacterial canker, and he stated that he had about two hundred trees of that variety ; of these he had lost four in 1927 and at the time of writing forty others showed yellowish foliage. On a visit to the plantation a few days later it was found that the grower's estimate of the damage caused was not exaggerated, for at least twenty-five per cent. of the trees bore unhealthy-looking foliage. All trees with these symptoms showed the typical cankered areas on the stems, and pieces of bark taken from a number of the cankers each contained masses of bacteria. Culture plates were prepared from particles of diseased tissues taken from two trees and in both cases an organism was isolated which proved to be similar to that obtained from trees at East Malling.

Experimental plots at East Malling have lost many trees as a result of this disease. An account of the trees, mostly Victorias, killed on one plot has already appeared (2). On another plot, consisting of Czars (187 trees), Utility (34 trees) and Purple egg (34 trees), nineteen (or ten per cent.) Czars were killed, or were so badly infected that they had to be removed, during the years 1928 to 1930, while fifteen others had cankers on branches, at the crotch or at the base of the stem but not girdling. On this plot trees of the other two varieties have not developed any cankers. On an adjoining plot of fifty-one trees of the damson Bradley's King, thirteen trees (twenty-five per cent.) were killed or severely cankered during 1928 to 1930.

The disease appears to be generally distributed in this country. Specimens showing the typical symptoms of bacterial canker have been received not only from several localities in Kent, but also from Surrey, Sussex, Hampshire, Dorset, Buckinghamshire, Gloucestershire, Worcestershire, Lincolnshire, Cambridgeshire and Cumberland.

Most of the varieties of plum generally cultivated in this country appear to be susceptible to bacterial canker. At East Malling those which have been most severely attacked are the three varieties mentioned above, viz., Victoria, Czar and Bradley's King. Other varieties on which the disease has been noted are Burbank's Giant Prune, Golden Drop, Warwickshire Drooper, Bastard Victoria, Prince of Wales, Farleigh Cluster damson, President, Rivers' Early Prolific, Evesham Wonder, Pershore Egg, Purple Egg, and Denniston's Gage. The author has not yet seen bacterial canker on Utility.

BACTERIAL CANKER IN NURSERY TREES.

During the summer of 1927 many " maiden " plum trees in nursery rows at East Malling were seen to be affected with bacterial canker and they presented an opportunity for comparing the symptoms on these young trees with those shown by older trees in plantations. On the maiden trees the symptoms were very similar to those already described, except that the effect was more intensified by reason of the thinner stems which were more readily girdled.

These young trees were mostly Victorias and Czars which had been " worked low " (budded or grafted) in the summer of 1925 ; they had grown vigorously during 1926, the stems being mostly about six feet long at the end of that season, and nearly an inch in thickness at the base. The stems were pruned back to about four feet.

During 1926 the leaves of many of them were badly infected with bacterial leaf-spot, and it is suspected that this might have had some connection with the serious outbreak of bacterial canker the following season. The relation that these two forms of disease bear to one another has not been determined, however, although it is now known that both are caused by the same organism.

The discoloured bark at the upper and lower limits of these cankers contained masses of bacteria, and isolations yielded a pathogenic organism similar to that found in cankers of older stems.

When examined during the last week of May, 1927, the healthy trees bore a head of long shoots which had developed from the upper buds, while the buds lower down the stem had produced short shoots. Infected trees at this stage showed the disease in various degrees of intensity and the following categories were noted :—

(*a*) A short canker on one side of the stem, the tree otherwise resembling a normal tree.

(*b*) A longer length of stem with dead buds or a definite canker present ; at this stage it was impossible to determine the limits of the cankers with certainty, without cutting into the bark ; leaves green and apparently normal.

(*c*) As in (*b*) but leaves definitely yellowish and mostly smaller than normal, indicating that the cankers had probably girdled the stems.

(*d*) Upper part of stem bearing wilting leaves and shoots.

(*e*) Upper part of stem with withered spurs.

(*f*) Upper part of stem with buds that had burst and then withered without growing out.

(*g*) Upper part of stem with dead buds which had not burst.

(*h*) Scion quite dead, but buds had grown out as short shoots before withering ; shoots growing out from the stock.

(*i*) Scion quite dead ; the buds had swollen before withering ; shoots growing out from stock.

(*j*) Scion quite dead ; buds not swollen ; shoots growing from stock.

(*k*) Whole tree dead, but buds had swollen ; no shoots on stock or scion.

(*l*) Whole tree dead, buds not swollen ; no living shoots on either stock or scion.

The cankered areas on the trees included under (*a*) to (*e*) showed up because of the dead buds on those areas. At the time the observations were made the other buds on the stem had grown into shoots of various lengths and the lesions with their unopened dead buds were therefore readily distinguished. On trees in categories (*f*) and (*g*), the lower limits of a lesion could be found immediately below the lowest dead bud.

On the trees which were infected and bore yellow leaves in May, those parts in the region of the cankers and above them died during the course of the season, the foliage wilting and remaining withered on the trees.

As in the case of the older trees, the cankers on these maiden trees did not increase in size during the summer, so that, where they did not extend to the base of the stem, the lower buds (which would have remained dormant or would have grown out into short shoots only) were stimulated in their development, and those buds immediately below a canker grew out into long vigorous shoots with normal green leaves, thus forming a striking contrast with the dead or dying shoots above the cankers.

INFECTION ON LEAVES, SHOOTS AND FRUIT.

In plantations where bacterial canker is rife, trees which do not show the characters described above may have other disease symptoms. Trees bearing foliage of the normal green colour often show circular dark spots on the leaves ;

these spots eventually become separated from the healthy tissues and, falling away, leave " shot-holes." In seasons when leaf infection is severe almost all the leaves of young trees may show such perforations. If the tissues of young developing spots are teased out in water, and examined microscopically under a low power, a gelatinous mass is seen to ooze out ; under higher magnification this is resolved into innumerable bacterial rods. The organism isolated from such spots has proved to be indistinguishable in culture from that isolated from the bacterial cankers of the stems ; moreover, it has been shown, by inoculation experiments on branches and stems, to be able to produce cankers.

In 1926, when bacterial leaf spot was particularly severe on Victoria plum trees on a plot at East Malling, lesions were also found in the bark of young green shoots and on the green fruit. On the shoots the lesions were elongated blackened patches up to 1.4 cm. in length. The lesions on the fruit were circular dark spots, the largest being about 2 mm. in diameter. Usually there were one or two spots only on each infected plum, but on one fruit there was a group of five spots. The infected fruits were relatively very few in number and no appreciable damage was caused to the crop from such direct infection of the fruit.

That is the only occasion on which the author has seen bacterial fruit spot in plums. The lesions on young shoots are of more frequent occurrence, however, and when they occur on young trees may be severe enough to kill the shoots. In such instances the effect resembles that caused by infection with *Pseudomonas prunicola*, described in an earlier paper (14).

It is of interest to note in this connection that the infection of green organs (leaves, shoots and young fruit) occurs as the bacteria in the stem cankers are dying out. Thus the organism passes the winter in the tissues of stems and branches, and the summer in the tissues of organs produced that year.

MONTHLY INOCULATIONS.

When it was found that plum trees could be infected by inoculations made in the autumn it was decided to carry out series of inoculations throughout the year to determine which were the most critical months for infection. Shortly before these experiments were carried out Brooks and Moore (6), working on the Silver Leaf disease, had shown that " there are striking differences in the chances of infection by *Stereum purpureum* in nature at different times of the year." It was thought likely that infection by the bacteria might show similar results particularly in view of their arrested development in the cankers during summer.

The inoculations were started in November, 1927, and continued until November, 1928. Three series of inoculations were carried out during this period.

Series A consisted of inoculations on two-year-old trees in nursery rows ; each tree was inoculated at two places on the stem and two trees were inoculated each month. The results given in the table were recorded in October, 1929.

Date of Inoculation.	Range in length of lesions in cms.	Average length of lesions.	Remarks.
1927.			
Nov. 11th ..	10—25	16.5	Three cankers girdled ; the fourth although 25 cm. long only half girdled the stem.
Dec. 9th ..	4—9	6.4	Stems ½ to ⅓ girdled.
1928.			
Jan. 11th ..	3—14	9.5	Stems ½ girdled.
Feb. 10th ..	2—4	2.6	Stems ⅓ girdled.
March 9th ..	2—3	2.6	Stems ¼ to ⅓ girdled.
April 5th ..	1—1.5	1.1	Three cuts healed normally ; the fourth showed slight infection.
May 11th ..	1.0	1.0	The cuts healed normally becoming covered with callus without any increase in the size of the wounds.*
June 11th ..	1.0	1.0	
July 11th ..	1.0	1.0	
Aug. 8th ..	1.0	1.0	
Sept. 12th ..	1—1.5	1.1	Three cuts healed normally ; the fourth showed slight infection.
Oct. 11th ..	2.5—6.5	3.6	Stems ¼ to ⅓ girdled.
Nov. 9th ..	5—56	25.0	One lesion girdled ; two ½ girdled. The fourth though 56 cm. long ½ girdled for most of its length and the girdling was almost complete in the middle region.

* The total length of the lesions is given by the figures. When a lesion is given as 1.0 cm. this indicates the length of the original cut and shows that no infection occurred.

Series B and C were inoculations made on the same dates as series A, but on young woody twigs. In each of these two series two twigs were inoculated, each at one place, monthly. The results were comparable with those of series A but the lesions were much smaller. The twigs inoculated in November, 1927, were killed outright, making no growth in 1928 ; lesions from inoculations in December and January were 2 to 3 cm. long and about half girdled the twigs, while those from the February to May inoculations were 1 to 1.5 cm. long. The inoculations made from June to August became completely covered by callus without any infection, the cuts being about 0.5 cm. long. The September inoculations resulted in a slight disturbance at one cut only, those of October gave lesions 1.5 to 3.5 cm. long, half girdling, and those of November (1928) 1.5 to 5 cm. half girdling.

The same strain of the organism was used throughout these experiments without re-isolation, and as the results obtained from inoculations in November, 1928, were, on the whole, of the same order as those of November, 1927, it is concluded that the negative results from the summer inoculations are due to a natural resistance of the stem during that period.

Other series of monthly inoculations were carried out in 1928-29 and 1929-30 and although the results were not so striking as those carried out previously they confirmed those results, showing that the most critical months for infection are October to December and that little or no infection occurs from the middle of April to the middle of September.

It should be remarked that all these inoculations were on stems or on woody twigs. Inoculations on young green shoots of the current year have yielded positive results in the summer.

THE CAUSAL ORGANISM.

A detailed description of the cultural characters of the organism causing bacterial canker in plum trees, and of the inoculation experiments which provided proof of its pathogenicity, will be given in a later paper, but it may be stated here that the bacterium is a white *Pseudomonas*, closely related to *Ps. prunicola*, which has been shown to be the cause of a bacterial shoot wilt of plum trees (14). Like *Ps. prunicola*, its colonies on nutrient agar containing five per cent. saccharose show radial lines, but it differs from that organism in certain other cultural characters, one being that it produces acid more readily in media containing sugar. Thus streak cultures on nutrient agar containing five per cent. saccharose become acidified so rapidly that transfers taken from such streaks, when seven days old, fail to give any growth, denoting that the organism is dead, presumably by reason of the acid which it has itself produced. On purple lactose agar containing two per cent. lactose there is first a reaction in the alkaline direction, followed by an acid reaction, so that the whole medium eventually becomes yellow. *Ps. prunicola* growing on this medium produces an alkaline reaction (shown by the indicator becoming blue-purple) which persists. In nutrient broth with glycerol (two per cent.) the canker organism produces a definite acid reaction.

The group number of the organism (indicating its salient characters) has been determined as 211.2222032. The figure .02 denoting the production of acid from lactose has been recorded for this organism by reason of its marked acid reaction when growing on purple lactose agar containing two per cent. lactose. It should be remarked, however, that when it is growing in nutrient broth containing one or two per cent. lactose the acid produced does not always completely neutralize the alkalinity resulting from the action on the broth itself, and the culture liquid may give a slightly alkaline reaction when compared with the uninoculated medium in control tubes.

Since it would appear that the bacterial canker organism has not previously been described, the name *Pseudomonas mors-prunorum* is proposed for it.

DISCUSSION ON CONTROL MEASURES.

Bacterial diseases of a virulent nature are generally difficult to control and the one under consideration will probably be no exception. Field observations and inoculation experiments, however, have provided information, relative to the behaviour of the organism in the field, which serves as a basis for exploring possible avenues in a search for effective control measures. Certain aspects of the problem are here presented for the consideration of growers and others who are interested in the disease.

Orchard Hygiene. In considering measures for the control of the diseases included under the term "die-back" it is essential that the primary cause of the trouble in each case should be adequately diagnosed. If the trees are unhealthy because of unsuitable soil conditions, attempts must be made to modify those conditions by drainage or cultivation. Again, if, as the writer believes, most cases of so-called die-back are a result of bacterial canker, the treatment will be very different from what it would be assuming that the cause of the trouble is a fungus such as *Diaporthe perniciosa* or a species of *Cytospora*. If one of these fungi could be proved to be the primary parasite then rigorous sanitary measures, involving the removal and burning of the dead trees during summer, before the fungus begins to produce fructifications freely, would probably be effective. Actually, however, the problem would appear to be far more complex if it is assumed, as the evidence here presented would justify, that the chief cause of die-back of plum trees in this country is a bacterium which spends about half the year in the stem and the other half on the leaves. Moreover, by the time the affected trees show the symptoms at all conspicuously the organism is no longer alive in the tissues of the stems, so that the removal and burning of diseased trees even as early as July is unlikely to be effective in reducing infection.

If infected trees are removed as a precautionary measure, to prevent infection spreading from them to other trees, the operation should be carried out as soon as the symptoms can be recognized. The object of this is to prevent those bacteria which are still alive in the stem lesions from reaching the leaves of that or of other trees. Whether such cankers are really a source of infection for the foliage has not been proved, but it has been found that leaf spots may appear on a tree before the bacteria die out in the canker. It is not certain how the initial leaf spots arise, but such observations suggest that they may be caused by bacteria from cankers on stems or branches, the transmission being possibly brought about by insects, or, when the cankers are on the branches, by rain.

It has been shown that infection of the stems and branches readily occurs through wounds made during the autumn and winter months, particularly October, November and December. It is essential, therefore, that trees should not be cut back or pruned during those months. To illustrate this point it may be mentioned that on two plum trial plots at East Malling it was necessary, in October, 1930, to cut back many of the plum trees in order to allow more space for pear trees with which they were interplanted. Although the pruning wounds were covered with a coating of white lead paint many branches died back or showed the typical symptoms of bacterial canker during the summer of 1931. In every case a definite cankered area was found towards the base of the affected portion, and on the canker, usually near the middle, there was almost invariably an obvious wound where a branch has been cut off. It is highly probable, therefore, that these pruning wounds served as ports of entry for the parasite.

Spraying. Spraying trials are on hand based on the knowledge acquired concerning the behaviour of the organism in the field. They have not yet yielded results sufficient for the recommendation of any particular methods with any degree of confidence, but it may be of interest to growers to know the object of the trials in case they would like to make similar attempts at control in their own plantations.

Since the organism passes the summer on the foliage it is reasonable to assume that if the leaf spotting could be reduced there would be less risk of stem infection in the autumn. One series of experiments has for its object, therefore, the control of this leaf spot stage by spraying with Bordeaux mixture at intervals during the summer. The experiments on these lines up to the present have been carried out in years when the incidence of leaf spot on the control trees was not sufficiently severe for any conclusion to be drawn as to the efficacy of the treatment, and they will be repeated. Summer spraying, if effective, would be particularly applicable to nursery trees.

Autumn spraying is also being tried. Since the cankers arise from infection in the autumn it is suggested that if the stems are coated with a bactericide just before, or about the time of incidence of infection, such treatment might ward off attacks. Trials are on hand, therefore, to test whether spraying the crotch and stem thoroughly with Bordeaux mixture in October and November will have any effect in reducing the number of stem cankers.

Resistant Varieties as "Stem Builders," or as Framework for Other Varieties. Another aspect of the problem is the possibility of raising resistant stems which could be worked high with a more valuable but a more susceptible variety. Experiments have been carried out on stems of Denniston's Gage (a variety which has rarely shown infection in the plantation) worked high with Victoria. Such stems, when artificially inoculated with the organism, developed cankers comparable in size with those developing on similar, but low worked, trees with Victoria stems. Whether a similar result would be obtained if the trees were left to natural infection is not yet known.

In taking field observations on Victoria trees some worked high, others low, on Myrobolan stocks, it was found that a greater percentage of trees worked low (i.e., Victoria stems) became infected than of those worked high (Myrobolan stems), and that on those trees with Myrobolan stems which became infected the canker in every case extended from the base of the branches downwards for some distance into the stock, suggesting that the infection started in the scion and not in the stock. Such trees, however, were so severely infected that they had to be destroyed and the method would appear not to be of any great practical value. It is thought, however, that if a resistant " stem-builder " is allowed to branch, and the grafting or budding performed on the branches and not on the main stem, it may be possible to produce a tree which would be less liable to stem infection. Experiments are being planned to test this. It is a method which has been tried with some success in America in attempts to combat Fireblight of pear trees, another bacterial disease.

Soil Conditions. Bacterial Canker occurs on trees grown on a variety of types of soil and at present it is not known whether the soil conditions can be modified in any way so as to reduce liability to infection. The possibility that the degree of susceptibility to this disease might be decreased by special manurial treatment has been considered and preliminary trials on plots of nursery trees have been carried out. These have not yielded any significant results, however, and further trials by pot cultures and field plots are contemplated.

SUMMARY.

1. Bacterial Canker of plum trees in plantations is characterized by:
   (*a*) the pale green or yellowish foliage of the trees in spring ; the leaves are often " rolled " ;
   (*b*) withering of the foliage during the course of the summer ;
   (*c*) the appearance of a cankered area on the stem, or on one or more branches.
2. The symptoms on young nursery trees are similar but they are usually more pronounced, the more severely infected trees making no growth whatever during the season.
3. In spring and early summer bacteria are found in dense masses in the tissues of the bark at the upper and lower extremities of the cankers.
4. During the summer the further development of the canker is arrested ; the bacteria present die out and become replaced by various fungi. Meanwhile bacterial lesions may appear on leaves (bacterial leaf-spot), shoots and fruit, of trees which do not show stem cankers.
5. Monthly inoculations on stems have shown that the critical months for infection are October, November and December ; inoculations from May to August failed to induce cankers.

6. The organism appears to be one previously undescribed, so the name *Pseudomonas mors-prunorum* is proposed for it.
7. Possible methods for controlling the disease are discussed.

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EXPLANATION OF PLATES.

FIG. 1.

A healthy Victoria plum tree in early summer. Note the long terminal shoots and compare with Fig. 2.

FIG. 2.

A Victoria plum tree with a Bacterial Canker on the stem. Note arrested development of terminal shoots, and the " thin " foliage. Figs. 1 and 2 represent trees of the same variety and age, growing on the same plot and photographed on the same day (June 28th).

FIG. 3.

The tree shown in Fig. 2, but four weeks later; by this time the leaves were completely withered.

FIG. 4.

A Victoria plum tree with a Bacterial Canker on one branch only.

FIG. 5.

A Czar plum tree with Bacterial Canker. Note the pale foliage, which at that time was yellow.

(Photograph taken on a Panchromatic Plate with a yellow screen to give correct tone value.)

FIG. 6.

Plum shoots (var. Bastard Victoria). Left from healthy tree. Right from a tree with Bacterial Canker. Note the narrow " rolled " leaves on the shoot from diseased tree.

FIG. 7.

A portion of the bark cut away at the edge of a canker early in spring. The sharp contrast between healthy and infected cortical tissues is shown.

FIG. 8.

Upper end of a canker in summer. Some callus has been formed and the bark is beginning to crack at edge of canker. Fungal fructifications are present on the cankered area.

FIG. 9.

A long canker which did not girdle the stem. The canker is bordered by well developed callus (for further description see text).

FIG. 10.

Maiden plum trees. The one on the right is healthy; that on the left is dead above and has a canker in the middle region of the stem where the buds failed to grow out.

FIG. 11.

Bacterial lesions on plum shoots and leaves.

FIG. 12.

Bacterial lesions on a young plum fruit.

(*Received* 12/10/31.)

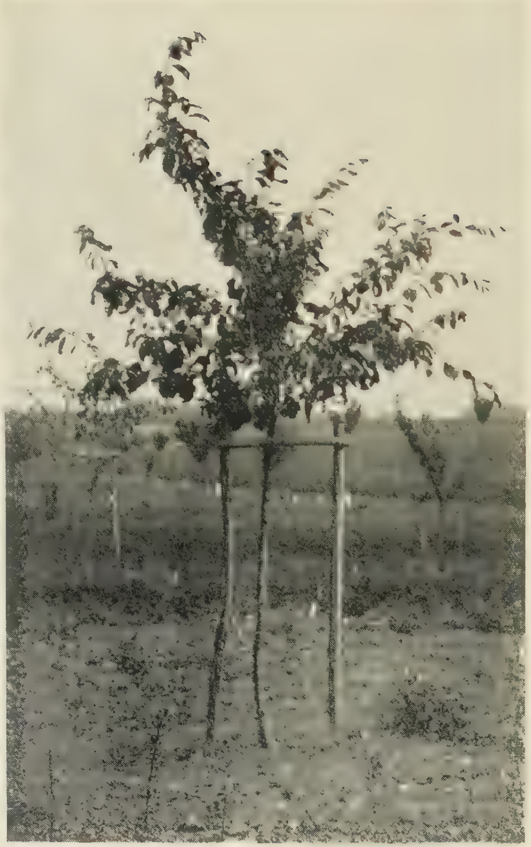


FIG. 1.

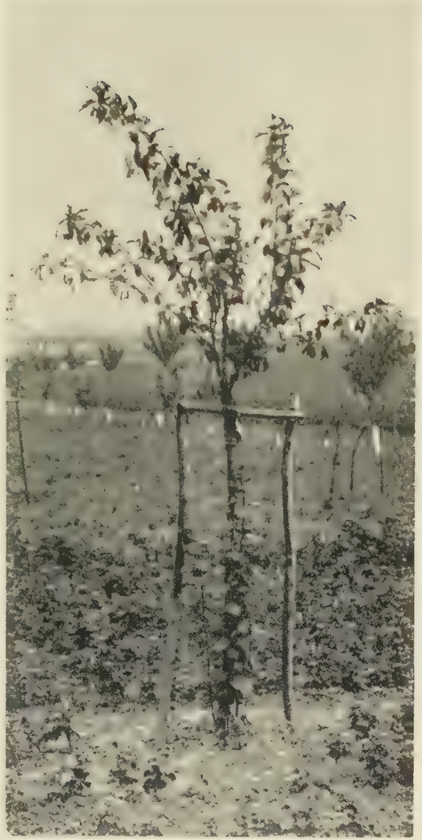
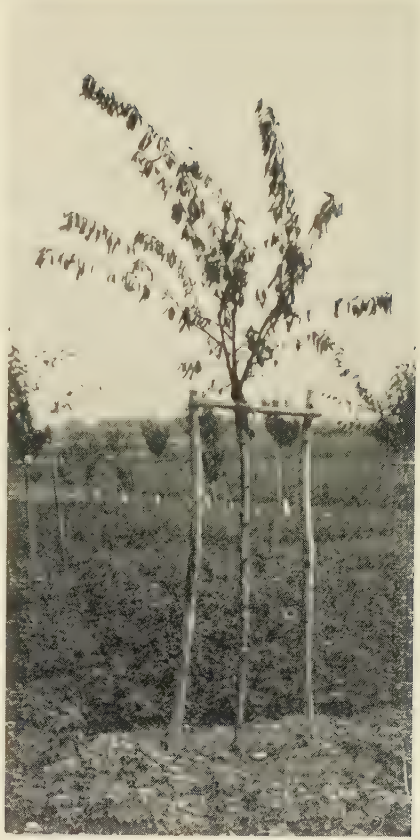


FIG. 2.



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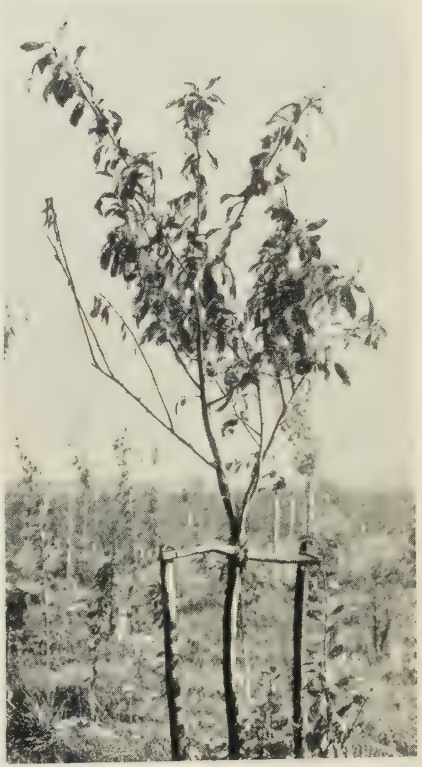

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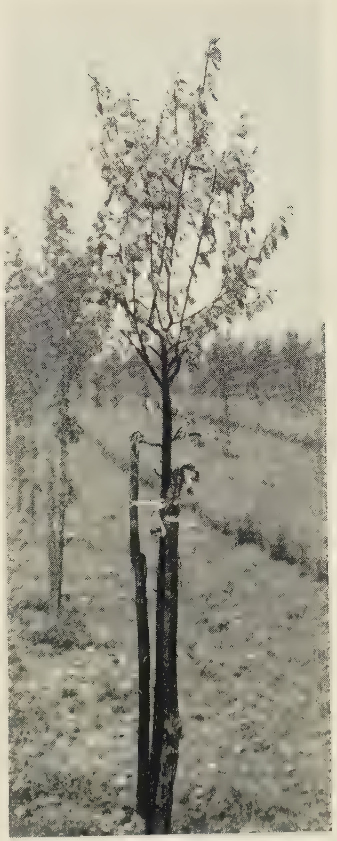

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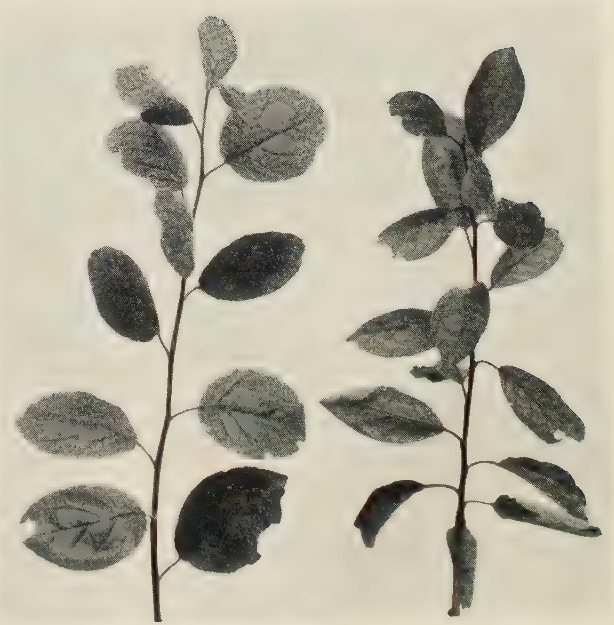

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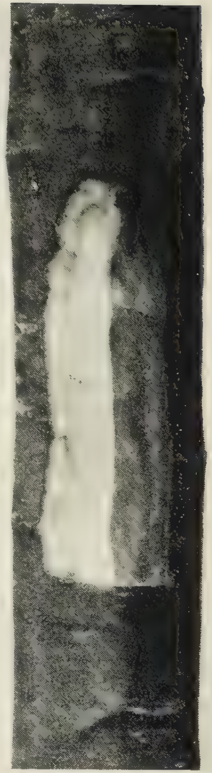

Fig. 7.

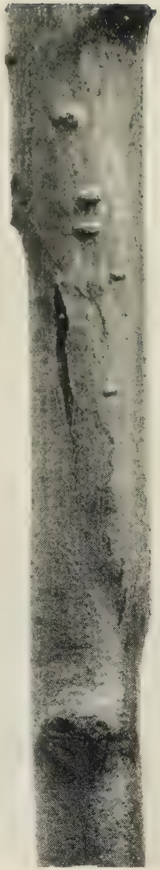

Fig. 8.

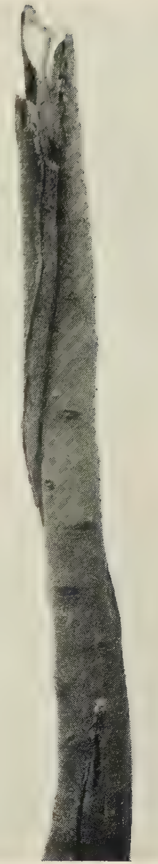

Fig. 9.

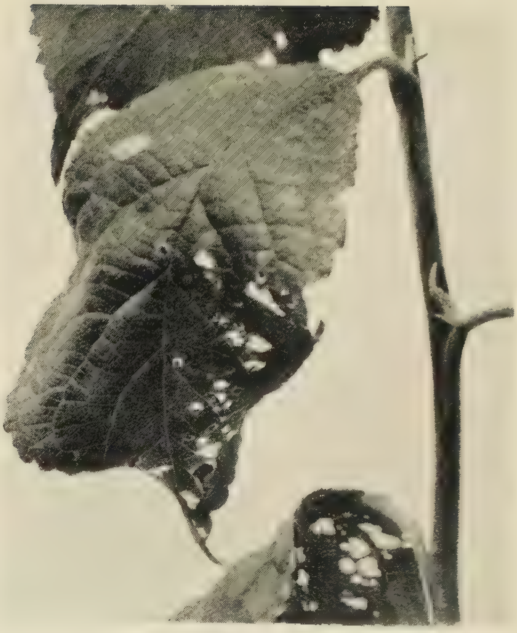

FIG. 11.

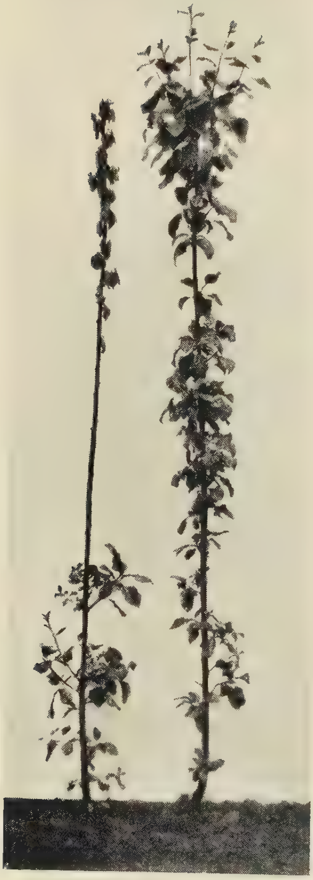

FIG. 10.

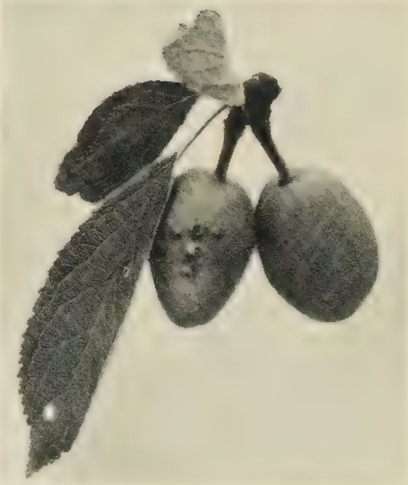

FIG. 12.

THE USE OF AMMONIA AND AMMONIUM SALTS FOR THE PREVENTION OF GREEN MOULD IN CITRUS.

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THE prevention of fungal rotting of stored fruits by the introduction into the atmosphere of substances which inhibit mould growth is in principle very simple. For the success of this method there are two essentials. The substances used should inhibit mould growth at concentrations which do not injure the fruit. If the substances are absorbed by the fruit they should not be injurious to health, or they should be chemically transformed within the fruit to innocuous products.

An example of a substance which fulfils these requirements and can be used for this purpose is acetaldehyde. An account of experiments demonstrating the value of acetaldehyde has already been published (1).

Ammonia is a substance which, when present in small quantities, has been found to inhibit mould growth on artificial media (data unpublished). It is also present in low concentrations in the animal body and there would, therefore, seem to be, prima facie, no objections to fruit containing small quantities, though this is a point on which we are not, of course, competent to express an opinion. The experiments recorded below were undertaken to discover if there are any definite concentrations in which ammonia might be used for preventing rotting of fruit in storage. The results show that at certain concentrations rotting is controlled without injury to the fruit.

METHOD.

The method employed was similar to that used to test the value of acetaldehyde. The fruit was stored in large glass desiccators in a continuous current of air. Before entering the desiccators the air was drawn through a tower of beads, down which dripped ammonia solutions. In this way atmospheres of controlled ammonia content were obtained.

The ammonia solutions used in the towers were formed by adding 100, 50, 25, 10 ccs. respectively of 0.88 ammonia to 10 litres of water.

These solutions were 0.176 N, 0.088 N, 0.044 N and 0.016 N respectively.

The volume concentrations of ammonia (ccs. per 10,000 ccs.) in the air current leaving the towers were found by estimation (absorption in hydrochloric acid) to be as follows:—

Temperature.	100	50	25	10 ccs. 0.88 ammonia per 10 litres of solution.
18° C. ..	20	10	5	2
10° C. ..	12½	6	3	1¼
3° C. ..	10	5	2½	1

The concentration of ammonia in the air was independent of the rate of current within the limits used, i.e., 1½-9 litres per hour.

STORAGE OF ORANGES IN CONTAINERS VENTILATED WITH AIR CARRYING SMALL REGULATED AMOUNTS OF AMMONIA.

Wounded and inoculated oranges have been used in all the storage trials.

Each orange was wounded sixteen times by picking it four times with a set of four pins stuck in the form of a square through a large cork. They were inoculated by spraying with a watery suspension of spores of *Penicillium digitatum* and were allowed to dry for an hour or so before storage.

For a preliminary trial samples of twenty oranges were stored at 18° C. The numbers attacked by *P. digitatum* after seven and fourteen days are recorded in Table I.

TABLE I.

Numbers Wasty in Samples of 20 Wounded and Inoculated Oranges stored at 18° C. in Presence of Ammonia.

Concentration of sol. (ccs. 0.88 Ammonia per 10,000).	Concentration in air (ccs. Ammonia per 10,000).	Days.	
		7	14
0	0	18	20
5	1	15	16
10	2	18	19
25	5	9	11
50	10	2	4

Ten and five parts per 10,000 of ammonia in the atmosphere were sufficient to check wastage to a considerable extent. The appearance and taste of the oranges kept at these concentrations were unimpaired.

Table II records a similar experiment using South African oranges. Samples of thirteen wounded, inoculated oranges were used. Two and five parts ammonia per 10,000 in the atmosphere reduced wastage considerably, without impairing appearance of flavour. Twenty parts per 10,000 caused considerable damage to the fruit in fifteen days. With ten parts per 10,000 there was slight damage to the skin of two oranges, but none to the taste.

TABLE II.

Numbers Wasty in Samples of 13 *Wounded and Inoculated South African Oranges stored in the Presence of Small Concentrations of Ammonia at* 18° *C.*

Concentration of Solution (ccs. 0.88 Ammonia per 10,000).	Concentration in Air (ccs. per 10,000).	Days.			
		7	15	21	28
0	0	11	13	—	—
10	2	4	7	8	9
25	5	3	4	6	6
50	10	5*	6*	8	—
100	20	1	1	—	—
Solid Ammonia Bicarbonate	—	2	2	4	7

* Desiccator flooded by Ammonia Solution.

While the presence of ammonia reduced the attack of *P. digitatum* it is interesting to record that it resulted in the development of *Colletotrichum* spots after twenty-one days. Two of the sound oranges remaining after twenty-one days in the presence of 5/10,000 ammonia showed *Colletotrichum* spots, and seven of those stored in the presence of bicarbonate showed similar spots after the same period.

The susceptibility to *Colletotrichum* attack in the presence of ammonia is of both theoretical and practical interest. Shipments of South African oranges have on rare occasions shown exceptionally high percentage wastage from damage by *Colletotrichum*.

The effects of temperature are shown by the results of the experiments given in Table III.

Less ammonia is needed for the control of *P. digitatum* with lower temperatures of storage. At lower temperatures also, less ammonia can be tolerated by the fruit. At 10° C. and 3° C. one part per 10,000 was sufficient almost completely to control rotting by *P. digitatum*. Three parts caused damage to the skin immediately surrounding wounds, while in the presence of ten to twelve parts the damage to the skin was general.

In order to show the relative efficiency of ammonia and acetaldehyde in controlling wastage by *P. digitatum*, Table IV is included. Comparing the two tables it may be seen that the margin between the concentrations needed for effective control and the concentrations which cause damage to the fruit is much the same in both cases.

TABLE III.

Numbers Wasty in Samples of 20 Wounded and Inoculated Oranges stored in the Presence of Ammonia.

Temperature.	Concentration of sol. (ccs. 0.88 Ammonia per 10,000).	Concentration in air (ccs. per 10,000).	Days.						
			9	16	23	38	53	61	73
18° C.	0	0	20*	—	—	—	—	—	—
	10	2	19*	19	19	—	—	—	—
	25	5	15*	15	16	—	—	—	—
	50	10	15*	15	15	—	—	—	—
	100	20	4*	4	4	—	—	—	—
10° C.	0	0	—	5	13	13	13	13	13
	10	1¼	—	—	—	—	1	1	1
	25	3	—	—	—	—	—	—	—
	50	6	—	—	—	1	1	1	1
	100	12½	—	—	—	—	—	—	—
3° C.	0	0	—	—	—	3	3	6	8
	10	1	—	—	—	—	—	—	—
	25	2½	—	—	—	—	—	—	—
	50	5	—	—	—	—	—	—	—
	100	10	—	—	—	—	—	—	—

* The suction failed from 2nd-5th day, during which time the concentration of Ammonia was not maintained.

TABLE IV.

Numbers Wasty in Samples of 20 Wounded and Inoculated Oranges stored in Air with Small Concentrations of Acetaldehyde.

Temperature.	Concentration of Solution (ccs. per 10,000).	Concentration in Air (ccs. per 10,000).	Days.						Condition of Remaining Oranges.
			7	14	20	30	40	60	
18° C.	0	0	20	—	—	—	—	—	
	25	20.8	11	11	13	14	—	—	No spotting of sound.
	50	41.6	0	3	4	4	—	—	Damage at Wounds, otherwise sound.
	75	62.4	—	2	4	4	—	—	Badly spotted at wounds.
	100	83	—	—	—	—	—	—	Severely spotted.
10° C.	0	0	—	17	18	18	18	19	
	25	11	—	—	—	3	5	6	Sound.
	50	22	—	—	1	1	2	4	Wounds obvious.
	75	33	—	—	1	1	1	1	Badly spotted.
	100	44	—	—	1	1	1	1	Badly spotted.
3° C.	0	0	—	—	—	—	—	15	
	25	7	—	—	—	—	—	0	Sound.
	50	14	—	—	—	—	—	1	Slightly spotted.
	75	21	—	—	—	—	—	2	Badly spotted.
	100	28	—	—	—	—	—	—	Badly spotted.

STORAGE IN THE PRESENCE OF AMMONIUM SALTS.

The dissociation pressures of certain ammonium salts are sufficiently great to give a strong smell of ammonia. Experiments have shown that ammonia is present in the air over such salts in sufficient amounts to inhibit the growth of moulds on artificial media.

Storage in the presence of ammonium salts has been tested, and Tables V, VI and VII record results of experiments.

TABLE V.

Numbers Wasty in Samples of 20 Wounded and Inoculated Oranges stored at 18° C. in Currents of Air passed through Bottles containing Various Substances.

	Days.		
	11	19	26
Air	18	—	—
Thymol	20	—	—
Ammonium Acetate	16	—	—
Aldehyde Ammonia	9	10	12
Ammonium Bicarbonate	2	4	4
Ammonium Bicarbonate (in Storage Chamber)	5	5	5

Table V shows that control of rotting by *P. digitatum* was not possible by passing an air current over thymol or ammonium acetate. Storage in an air current passed over aldehyde ammonia or crystals of ammonium bicarbonate largely prevented rotting. Aldehyde ammonia was not so effective as the bicarbonate. Moreover, the oranges finally remaining sound tasted of aldehyde. The bicarbonate was effective, whether placed in the bottom of the desiccator through which the air stream passed, or whether the current passed over it before entering the desiccator. Ammonium bicarbonate was also effective at 10° C. and 30° C. (Table VI).

TABLE VI.

Numbers Wasty in Samples of 20 Wounded and Inoculated Oranges stored in Currents of Air passed through Bottles containing Ammonium Bicarbonate.

Temperature.	Days.			
	20	33	48	71
10° C.	4	5	6	6
3° C.	—	—	—	—

Table VII records the wastage of samples of oranges placed in desiccators in the presence or absence of crystals of bicarbonate. The control of *P. digitatum* is effective even when the oranges are contained in their wrappers, and was approximately equivalent to that obtained when ammonia was present in two to five parts per 10,000 at 18° C.—concentrations at which rotting is checked without damage to the skin or interference with the taste.

TABLE VII.

Numbers Wasty in Samples of 10 *Wounded and Inoculated Oranges stored in Desiccators at* 18° *C.*

		Days.						
		7	11	14	22	31	38	44
No Ammonium. Bicarbonate.	Wrapped ..	10	—	—	—	—	—	—
	Not Wrapped	10	—	—	—	—	—	—
Ammonium. Bicarbonate.	Wrapped ..	—	2	4	4	5	6	6
	Not Wrapped	2	2	2	3	3	4	7

THE USE OF AMMONIUM BICARBONATE IN THE COMMERCIAL HANDLING OF CITRUS.

An interesting problem arising from the above experiments is whether the property of ammonium bicarbonate of checking wastage by *P. digitatum* can be utilized in the commercial handling of citrus.

The introduction of solid crystals into ships' holds, or land stores or railway waggons should present no difficulties, and trials of this nature could easily be carried out.

TABLE VIII.

Numbers Wasty in Samples of 10 *Wounded and Inoculated Oranges stored in Trays in the Open Constant Temperature Chambers.*

Temperature.		Days.						
		7	9	12	16	23	30	37
18° C.	A. ..	—	6	7	10	—	—	—
	B. ..	1	2	4	5	5	7	8
10° C.	A. ..	—	—	—	2	9	9	9
	B. ..	1	1	1	1	4	6	7

A.—Control. B.—A Crystal of Ammonium Bicarbonate included in the Wrappers.

The inclusion of crystals of bicarbonate in the wrapper is also an obvious and simple method. Trials have shown (Tables VIII and IX) that this method does not give such good results as storage in a current passing over, or in a desiccator containing, crystals of the salt. The reason for this is not obvious but may possibly be due to the different humidity conditions of the various sets of experiments. Ammonium bicarbonate does not dissociate when dry. In most of the experiments recorded the oranges were stored in glass desiccators in which the humidity was doubtless high and the ammonia present could be smelt. When oranges, wrapped with crystals, have been stored on trays in the open the crystals have remained in the papers and no smell of ammonia has been apparent. When oranges, wrapped with crystals, have been stored in desiccators the crystals disappeared, probably owing to the absorption of ammonia by the acid fruits, and with the disappearance of ammonia rotting took place. Effective control was only obtained for about five days.

TABLE IX.

Numbers Wasty in Samples of 10 *Wounded and Inoculated Oranges wrapped and stored in Desiccators in presence of Ammonium Carbonate and Bicarbonate. Laboratory Temperature.*

	Days.		
	5	9	16
Control	8	10	—
Ammonium Bicarbonate	1	7	10
Ammonium Carbonate	1	7	9

USE OF AMMONIUM BICARBONATE FOR THE CONTROL OF FUNGI IN THE STORAGE OF FRUITS OTHER THAN CITRUS, AND IN THE STORAGE OF OTHER PRODUCTS.

No attempt has so far been made to carry out storage tests with fruits other than citrus. Small samples of strawberries and raspberries develop moulds when stored in a jar in the presence of ammonium bicarbonate. On the other hand a few wounded grapes remained free from mould attack in a flask containing crystals of the salt. Trials with grapes will therefore be undertaken as soon as possible.

It is not only in the storage of fruit that losses from mould growth are encountered, but also in the storage of other food products, as for example, meat, eggs, butter and dried fruit, and such varied products as timber, leather, rubber, and woollen and cotton goods. There is no doubt that in many instances mould growth on these substances could be prevented by the presence of ammonium salts. So far experiments conducted with meat have proved unsuccessful. The reason for this appears to be that the meat is acid and that the concentration of ammonia is not maintained at the surface on which growth takes place. Ammonia would not be removed in this way by some of the other products, such as leather and textiles, and mould growth on them would therefore be prevented by the presence of small quantities of ammonia in the atmosphere.

SUMMARY.

Green rot of citrus due to *Penicillium digitatum* is largely reduced by storage in an atmosphere containing small regulated concentrations of ammonia. An effective concentration for this purpose is maintained by damp crystals of ammonium bicarbonate.

REFERENCE.

(1) *S. A. Trout* and *R. G. Tomkins*. "The Use of Acetaldehyde in the Storage of Fruit." Commonwealth of Australia Journ. of Council for Sci. Ind. Res. Vol. 4, p. 6, Feb., 1931.

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THE INFLUENCE OF VEGETATIVELY RAISED ROOTSTOCKS UPON THE APPLE, WITH SPECIAL REFERENCE TO THE PARTS PLAYED BY THE STEM AND ROOT PORTIONS IN AFFECTING THE SCION.*

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In early grafting practice the rootstock almost invariably comprised a root-system, which performed the functions of absorbing water and mineral substances from the soil, and a varying length of stem, which served as at least part of the " path " by which these materials were carried to the scion (bud or graft). At first this rootstock was thought only to serve a function quite subsidiary to the scion. Then another school arose which attributed an almost fantastic influence of the stock over the scion. Modern nursery practice in Europe was built up on the assumption that rootstocks could be used to determine to some extent the vigour and cropping of the subsequent tree and that certain scions and stocks were uncongenial. There was, however, little or no precise data published or available as to the actual extent of these influences. Although the subject had attracted one or two early horticultural investigators, the nature of the material (mostly seedlings or unidentified botanically), the small number of plants, and the elementary arrangement of plots employed combined to prevent the collection of any exact information. In parts of America, exigencies of climate and labour organization have brought into being the comparatively recent method of grafting directly on to the root—either the whole or "piece" roots. Rootstock influence has not generally been considered of primary importance in these regions. This has brought to the fore the interesting problems of the extent and causes of stock influence.

Review of Results with Stem Worked Rootstocks.

At the Meeting of the Agricultural Section of the British Association in 1920 (at Cardiff), the earliest indications of rootstock influence upon the two apple scion varieties—Lane's Prince Albert and Bramley's Seedling—were presented from East Malling (9). The rootstocks had been described botanically, raised vegetatively by layers, and were thus believed to be true clonal races. Although the trees worked upon these varieties of rootstock were only two years old, the numbers were quite considerable (420) and the pomological characteristics of growth and blossom formation so constant for any one combination of stock and scion that a preliminary grouping of the rootstocks under trial was hazarded. At two years old it was only possible to emphasize vigour—as expressed by total measurement of growth and weight of tree—and precocity in fruit bud formation, as expressed by the percentage of trees which had formed any fruit buds and the number of fruit buds per given length of wood growth, and actual set of fruit. These symptoms suggested the following grouping:—

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(a) Very dwarfing—Rootstocks	No. VIII (French Paradise) No. IX (Jaune de Metz)
(b) Semi-dwarfing	No. II (Doucin) No. V (Doucin Ameliore)
(c) Vigorous	No. I (Broadleaf) No. VI (Nonsuch)
(d) Very vigorous	No. XII (Layered " Free " Stocks) Nos. XIII to XVI ,, ,, ,,

These facts are here recalled because of the similarity of the new data to be presented later from comparatively young trees.

Six years later, at the British Association Meeting at Oxford, a much more considerable body of numerical data was presented, after careful statistical examination, showing that these early predictions had been very generally fulfilled and that in amount of total wood growth, height and spread of tree, girth of stem, habit of growth, blossom bud formation, percentage of blossom setting, time of blossoming, and actual number of fruits borne, there were striking significant differences. Such differences as five times the amount of growth and ten times the amount of fruit between trees of a single variety worked upon two different rootstocks, although otherwise treated exactly similarly (7), were demonstrated.

The Meaning of Rootstock Influence.

It was further emphasized that, apart from these easily measurable characteristics, there were certain manifestations of tree behaviour associated with the different groups of rootstock influence which were perfectly obvious to the pomologist, though difficult to record in precise terms, and which no single group name such as "semi-dwarfing" or "vigorous" could adequately express. Some of these characteristics have since been numerically measured, others still defy the statistician, but they are nevertheless an essential part of what the pomologist sees in " stock influence."

It is quite certain that uniformity of " stock influence " will never be appreciated by a solitary study such as the length of shoot growth on one and two-year-old trees. In many seasons the actual shoot growth in the first year of trees on the " Very dwarf " No. IX stock will exceed the total shoot growth on the " Very vigorous " No. XVI, but nothing has so far turned the potentialities of those on No. IX into becoming " Very vigorous " No. XVI's or vice versa. Again, in a nursery row of young trees on a single variety of stock there will be variation in amount of growth, and although in later life these trees will probably retain some measure of their relative vigour, they behave alike in all other respects. The " Very dwarf " group (a)—Nos. VIII and IX—have invariably ultimately produced trees of relatively dwarf, slender, spreading, bifurcating growth, with an exaggerated swelling at the point of union, a relatively poor anchorage, early axillary and spur fruit bud formation, a high percentage of flowers setting fruit when the tree is young, and in the early years exceptionally large well coloured fruits. Nos. II and V have always been described as (b) " Semi-dwarfing "—although many varieties are now known, under optimum conditions, to make as vigorous growth upon them as upon the " Vigorous " group (c) stocks, such as VI and I. But this " Semi-dwarf " group has its special propensity to comparatively early fruit bud formation and fruit set, a lesser swelling at the union than in trees on No. IX, and a moderate anchorage, probably due to the lack of fibrous root. A special propensity to " leaf scorch " has also been shown to belong to this group. Nos. I and VI represent the " Vigorous " group (c), where fruit bud formation is significantly later and fruit set less in the early years. The anchorage is good, the growth generally sturdy, and the stock and scion make a more or less perfect union. The " Very vigorous " group (d), represented by such stocks as XII and XVI, produces trees of immense vegetative vigour, which is not always obvious in the early years, with comparatively late formation of fruit buds and small percentage of blossom setting fruit. In the early years, these fruits are often of poor quality. The type of tree is distinct both in its upright and branched growth, in its sturdiness and perfect anchorage.

Evidence has been presented to show that between these very definite types, stocks may be found producing trees of intermediate behaviour. It has more recently been shown that individual scions and rootstocks may have particular affinities or the reverse and that special local environmental conditions may modify particular characteristics (8). It may even be added that time may alter the relative relationship betwixt such things as growth and fruiting, size and quality of fruit. However, twelve years' close observation of some 2,000 individually recorded trees, of a considerable number of varieties, and upon five different soils, goes to show that the influence of vegetative rootstocks does not lessen with time but must be closely studied where questions of nutrition (1), disease resistance (12), and storage quality (16), are being considered.

Examples of Practical Significance of Rootstock Influence.

Stock influence is thus of great practical value to the grower in obtaining a tree of special potentialities. Some idea of what it would have meant to him in the first eleven years of the apple tree's life is suggested by the following figures, which are based upon the performance of trees planted equidistant.

The suitable distances at which to plant are calculated for the average height and spread of the trees, i.e., the space they have occupied. In actual fact the performance might be slightly modified by such questions as root competition, though there is as yet no evidence to suggest this.

TABLE I.

Variety.	Stock.	Distance in feet.	No. of Trees per acre.	11 year total bushels per acre.
Bramley's Seedling ..	IX.	12	302	3,020
	II.	24	75	703
	I.	24	75	875
	XII.	36	33	284
Lane's Prince Albert ..	IX.	10	435	2,232
	II.	15	193	1,129
	I.	18	132	1,010
	XII.	24	75	382
Worcester Pearmain..	IX.	10	435	1,718
	II.	15	193	796
	I.	15	193	970
	XII.	20	109	414

The total amount of fruit borne by these three varieties on three rootstocks at the sixth and eleventh years gives a good idea of stock effect upon earliness of bearing.

TABLE II.

Fruits in lbs. per tree.

Variety.	Stock.	At 6 years.	At 11 years.
Bramley's Seedling	IX.	32	402
	II.	17	375
	XII.	1	344
Lane's Prince Albert	IX.	38	205
	II.	22	234
	XII.	3	204
Worcester Pearmain	IX.	15	158
	II.	12	165
	XII.	4	152

One more instance of the practical value of a knowledge of stock influence may be given in a comparison of the cropping capabilities of the true Doucin (No. II) and the so-called " Improved " Doucin (No. V), which in commerce has unfortunately, owing to its ready propagation, largely replaced the former stock.

TABLE III.

Fruits in lbs. per tree.

Variety.	Stock.	At 6 years.	At 11 years.
Bramley's Seedling	II. V.	17 9	375 277
Lane's Prince Albert	II. V.	23 16	234 183
Worcester Pearmain	II. V.	12 5	165 111

Nor is this the whole of the story, for the exceptional propensities of No. V to leaf scorch often so affect the size and colour of the fruit that much of it is hardly saleable.

The Theory of Rootstock Influence.

How, then, is this rootstock influence brought about ? Is it inherent in the stem portion of the stock—the " path " by which the materials collected by the roots find their way to the scion ? If so, it has been suggested that the laborious method of raising vegetative rootstocks could be abandoned, and the desired combination of qualities (termed " stock influence ") could be imparted by placing a piece of the selected stock stem upon a seedling root, as an intermediate between the root and the ultimate scion. Incidentally this practice of double-grafting is at least as expensive as stock layering and much more uncertain.

The Part Played by the Stock Stem—Evidence Reviewed.

Considerable evidence has already been published which proved that such an " intermediate " piece of stem placed betwixt rootstock and scion does exert an influence upon the scion. Knight began investigations along this line in 1921 (11), using as " intermediates " pieces of the stems of rootstocks No. IX (Very dwarfing) and No. I (Vigorous) and grafting each reciprocally as close to the roots of the other as possible and similarly upon its own roots also and finally top working the whole series with Lane's Prince Albert. Thus the series of trees, the units of which were admittedly small—only six—consisted of Lane's

Prince Albert with No. I as intermediate upon No. I roots ; No. I as intermediate upon No. IX roots ; No. IX as intermediate with No. IX roots, and No. IX intermediate with No. I roots.

The records of wood growth and blossom formation for three years led to the conclusion that the introduction of a piece of No. IX stem between the roots of No. I and the scion *considerably reduced the length of new wood growth,* after the first year and *materially increased the number of fruit buds* formed. Vice versa, the introduction of a piece of No. I stem between the roots of No. IX and the scion *consistently increased the length of new wood growth* and *slightly modified the number of fruit buds* formed. The number of blossom buds per metre of new stem was also affected by the intermediate stem, and these trees of Lane's Prince Albert with a different intermediate from the rootstock (IX on I and I on IX) exhibited a behaviour roughly midway between those worked on intermediates the same as the rootstocks (I on I and IX on IX).

Knight pointed out that the effect of the change of " intermediate " is much greater when the root system was vigorous (No. I) than when it was dwarfing (No. IX), and he concluded that " whilst the root system of the stock is probably the dominating factor in the influence of the stock upon scion, yet at the same time the stem of the stock also plays a part which may be considerable."

This series of trees, though it suffered several casualties, was observed for a further period of three years before it had to be grubbed, and, although the figures have not been published, Dr. Knight has permitted reference to them. Both in the case of annual length of new wood growth and the number of blossom buds formed per unit length of stem, the trees at six years old very generally confirm the early findings as to the influence of the intermediate stock stem, and the average total weight of the trees at lifting still further bears it out ; the Lane's Prince Albert trees on No. I rootstocks with No. IX intermediates being approximately half the weight of those with No. I intermediates.

Grubb's work with double-worked pears (3 and 4) and apples, certainly confirms the influence of the intermediate piece of stem. " The effect on the vigour of the trees of the different varieties used as intermediates is strikingly different." In the case of pears, he finds significant differences both in wood growth and blossoming. Although in the case of apples he had been using combinations of varieties of extreme difference and has seen definite though not striking results (5), the very interesting piece of evidence has in some cases been demonstrated, namely, that a greater length of intermediate may intensify an effect produced by a lesser one (8).

It is true that in all these instances a small piece of stock stem has been involved, but the point to be emphasized here is that *an intermediate piece of* *stem has a definite effect*, and, where that intermediate has been a rootstock variety, it has behaved in a modified form like the complete rootstock normally does.

All these pieces of evidence go to confirm the traditional ideas about double working and top grafting, where the intermediate has long been regarded as a " good or bad mother." At the same time it is probably true to say that nothing quite so striking as the extreme comparisons in the rootstock experiments has as yet become apparent.

Working in America from quite a different standpoint, with quite different material and technique, Roberts and Swarbrick shed an interesting new light upon the question. A common practice in America is to " bench graft "—during winter—the scion variety directly on to the true root of a seedling stock. Climatic exigencies favour this method and the subsequent growth conditions are so favourable that good maidens, such as are rarely if ever obtained by this method in England, result. American nursery tradition had long claimed that the scions thus grafted materially altered the seedling root, and the observations of these workers strikingly confirmed this. The fact that this influence had not been obvious on stem-worked trees in England led these investigators to make observations on young trees of from one to three years old both worked on to the root direct and stem budded high upon seedling stocks. Since scion influence upon the root was marked in the first case and not in the second, they were led to conclude that *the effect produced by the use of vegetative rootstocks " is a stem influence rather than a direct root influence "* (14). Again, the marked uniformity in growth of established orchards of trees bench grafted on to seedling roots in America pointed to the same conclusion. It would incidentally be interesting to know whether they are equally uniform in other characteristics such as type of growth, cropping capabilities, disease resistance, etc. After further experimentation, Roberts was far more emphatic in concluding *" obviously stock influence is due to the stem portion of the stock "* (13).

These workers point out that " It is significant to note that there are no records of what happens in the case of trees which have been grown directly upon roots which arise from vegetatively propagated rootstocks. It is, of course, common nursery experience that piece root grafts of lateral roots give poor stands " (14).

Records of such trees were not easily available for two reasons besides the one supplied above—bench grafting of any sort has never been considered a practical method of tree propagation by European nurserymen. Secondly, experimental attempts from 1923 onwards to establish such series of trees at East Malling had resulted in a large percentage of the scions establishing themselves on their own roots (2).

The Part Played by the Root of the Stock—Fresh Data Presented.

However, a new series of trees was planned in the autumn of 1927 and a different technique employed. The bench grafted plants were set out in the nursery with the unions above ground level to prevent scion rooting. In the first season the earth was, however, mounded up well above the point of union so as to prevent drying out, but was from time to time drawn away from the plant for examination. When the scions were well established, the soil was finally drawn away altogether, leaving a short length of root exposed at the point of union. This material, consisting of scions grafted both on to the roots and stems of stocks, was actually planted out in the nursery in January, 1928. It comprised the two varieties, Beauty of Bath and Grenadier, because it had previously been shown that, when used as scions on stem worked stocks, they exhibited some slight quantitative effect upon the relative amount of root and the relative amount of fibre on the rootstock (6). Three vegetatively raised rootstocks were used—Nos. II (Doucin) and VI (Nonsuch) because of the well marked differences in their morphological characters, and No. VIII (French) as representing the very dwarfing group of layered stocks. A further series of selected seedling free stocks, one year old, was included. Amongst the many combinations prepared were the following, which supply the data to be presented in the present paper:—

TABLE IV.

Numbers. Worked.	Numbers. Succeeded.	Variety.	Grafting Method.	Rootstock.
50	46	Beauty of Bath	On 1 year stem.	No. VI.
50	40	,, ,,	,, ,,	No. II.
50	12	,, ,,	,, ,,	No. VIII.
50	50	,, ,,	,, ,,	Seedlings.
50	26	,, ,,	On Piece Root.	No. VI.
50	23	,, ,,	,, ,,	No. II.
50	26	,, ,,	,, ,,	No. VIII.
50	47	,, ,,	On Crown Root.*	Seedlings.
50	46	Grenadier	On 1 year stem.	No. VI.
50	41	,,	,, ,,	No. II.
50	50	,,	,, ,,	Seedlings.
50	44	,,	On Piece Root.	No. VI.
50	20	,,	,, ,,	No. II.
50	45	,,	On Crown Root.*	Seedlings.

* The aim of grafting on to the " crown " of the seedling, i.e. as low as possible—was to eliminate the whole of the stock stem.

As will at once be seen, the mortality in many cases was considerable. This was due to several causes. First, in order to obtain as comparable material as possible, the inexpedient method of grafting one-year layers straight off the stool in January and planting them forthwith in the nursery had to be adopted. Nos. II and VIII are notoriously slow to callus and to recover from transplanting, and in both a high mortality and lack of uniformity of early growth resulted. Second, in order to obtain the necessary number of vegetative piece roots, some six-year-old stools had to be dug up and lateral pieces of different ages, sizes and, probably, potentialities for recovery and growth were used. The trees upon which observations of growth, blossoming and fruiting have been made, were, with a view to saving time and space, left in the nursery rows at one foot apart by three feet between the rows. All lateral shoots have been pinched back in summer to three buds and only the one main extension leader has been allowed to grow on untouched. This abnormal treatment has, of course, not permitted of a complete measurement of the annual growth being taken, and it has most probably affected the wood measurements of the different series, and masked possible significant differences therein, since most of the laterals on Nos. VIII and IX, as is known from ample previous experience, would normally develop axillary fruit buds on the current season's growth, whilst the laterals on No. VI would have mostly been strong wood shoots.

Prior to this treatment the maiden growth showed most significant differences. The Beauty of Bath trees root grafted on No. VIII averaged 37.9 cm. and those on No. VI 57.5 cm. Early axillary fruit bud formation was also apparent on the trees on No. VIII (10). Again, when at two years old 50 per cent. of the trees were lifted and weighed before root examination (15), the figures were as follows:—

	Worked on	
	Stems.	Roots.
Beauty of Bath on VIII ..	156 gr.	163 gr.
,, ,, II ..	211	253
,, ,, VI ..	207	190
,, ,, Seedlings ..	324	313

This again well illustrates stock effect on stem growth prior to any effect produced by the pinching out of lateral shoots. Despite this somewhat abnormal treatment, the different groups of trees have been quite unmistakable, as both the illustrations (see Figs. 1, 2, 3) and tables will show.

It is first of all possible to see what effect the rootstocks have had upon the two varieties when grafted on the stem at the normal height of three inches above ground level, and then to compare the behaviour of a similar series grafted directly on to a piece root.

All the series on selected one-year-old Seedlings have contained a big majority of vigorous trees, partly fulfilling traditional belief that a good seedling gives vigorous growth, and partly bearing witness to the fact that one-year seedling roots are more suitable to these particular forms of propagation than vegetative ones. On the other hand, in some instances they exhibited striking variations, especially in fruit bud formation.

TABLE V.

Beauty of Bath, four-year-old series.

Stock.	No. of trees.	Total Leader growth.	Girth at 10 cms. in cms.	Per cent. Blossoming at		Total Blossom Trusses per tree.	Per cent. Setting Fruit at		Total No. of Fruits per tree.
				3 yrs.	4 yrs.		3 yrs.	4 yrs.	
		Grafted on STEMS of 1 year layers.							
(a) Dwarfing VIII	6	167	6.1	67	100	34	17	100	6
(b) Semi-dwarfing II	27	162	6.4	81	96	15	7	63	2
(c) Vigorous VI	35	177	6.4	42	72	4	0	0	0
(d) Seedling	40	231	8.3	5	20	0.5	0	8	0.1
		Grafted on Piece ROOTS.							
(a) VIII	14	169	5.6	100	100	38	64	100	10
(b) II	11	213	7.8	90	91	15	36	73	3
(c) VI	33	198	6.8	42	85	6	3	9	0.4
(d) Seedling	35	219	7.5	11	31	1	0	6	0.06

Table V gives the data for the four-year-old series of Beauty of Bath. In the stem worked series there is up to the present no significant difference between the total leader growth on Nos. VIII, II and VI, probably due to reasons already suggested (i.e., the abnormal method of grafting one-year layers and the consequent high variability within each group) and to the small unit of trees on No. VIII surviving in the nursery row,* but the differences in character of growth, percentage of trees blossoming, total number of blossoms, percentage of fruit set, are for the most part highly significant.

Unexpectedly, in nearly every case these stock differences are not only maintained but emphasized where the trees have been grafted directly on to vegetative piece roots. Here even the leader growth differences between the trees on No. VIII and Nos. II and VI are significant. The average height of the trees on No. VIII, including this season's growth (1931), is 215 cms. as compared with 263 cms. on No. VI. In addition, the actual growth of the trees on No. VIII roots ceased at the end of July, whilst those on No. VI roots added, on the average, nearly 20 cms. new extension growth before the end of September.

Table VI gives exactly confirmatory data for a second series of trees a year younger, and with the "very dwarfing" rootstock No. IX substituted for

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* Since the above was written, a further year's measurements has now revealed a difference of some 15 cms. between the height of the trees on Nos. VIII and VI.

No. VIII. Whilst up to date there is the same irregularity in total leader growth in the stem grafted trees, the figures as a whole can leave no doubt about stock effect on blossoming and fruiting. The trees grafted on to piece roots are again the more emphatic.

TABLE VI.

Beauty of Bath, three-year-old series.

Stock.	No. of trees.	Total Leader growth.	Per cent. Blossoming at 2 yrs.	Per cent. Blossoming at 3 yrs.	Total Blossom Trusses per tree.	Per cent. Setting Fruit.	Average No. per tree.
		Grafted on Stems of 1 year layers.					
(a) IX ..	33	114	6	100	6	61	1.4
(b) II ..	30	89	0	63	3	23	0.3
(c) VI ..	26	105	0	12	0.1	0	0
(d) Seedling ..	45	159	0	2	0	0	0
		Grafted on Piece Roots.					
(a) IX ..	32	100	22	84	5	50	1.4
(b) II ..	33	128	16	81	5	27	0.8
(c) VI ..	39	122	5	33	1	0	0
(d) Seedling ..	26	142	0	0	0	0	0

Table VII shows the data collected upon a similar series of Grenadier—a variety altogether more prone to early fruiting, yet even here the story of the early cropping of Nos. IX and II as compared with No. VI is significant (see Plate II). A series of Grenadiers a year older, which unfortunately did not include a very dwarfing stock, still points to the difference in behaviour with trees on II and VI, those on the former having consistently more blossom trusses which set a higher percentage of fruit. These records on the influence of clonal piece

TABLE VII.

Grenadier, three-year-old series.

Stock.	No. of trees.	Total Leader growth. cms.	Per cent. Blossoming at 3 yrs.	Per cent. Blossoming at 4 yrs.	Total Blossom Trusses per tree.	Per cent. Setting Fruit.	Average No. per tree.
		Grafted on Stems of 1 year layers.					
(a) IX ..	28	100	14	93	12	89	6.6
(b) II ..	29	76	7	76	7	59	4.6
(c) VI ..	41	81	2	44	1	10	0.1
(d) Seedling ..	49	131	4	49	3	22	1.0
		Grafted on Piece Roots.					
(a) IX ..	32	102	34	94	12	91	5.6
(b) II ..	17	82	12	94	8	82	5.8
(c) VI ..	28	100	7	86	7	43	0.9
(d) Seedling ..	31	129	0	42	2	26	0.6

roots upon young trees so clearly coincide with those published eleven years ago from trees worked upon identical clonal stems that, in the absence of any definite evidence that the exposure of portions of the vegetatively raised stock root, as described above, results in their assumption of complete stem characteristics, it can be suggested with some confidence that the root of the vegetatively raised stock plays at least as great a part as the stem portion.

SUMMARY.

Information is here reviewed which proves the clonal races of vegetative rootstocks when stem worked influence the scion significantly, at least in the first twelve years, in the amount of total wood growth, the habit of growth, the height and spread of the tree, the stem girth, earliness and nature of blossom bud formation, time of blossoming, percentage of flowers setting, actual number of fruits borne, size, colour and quality of fruit, degree of susceptibility to diseases such as Scab and Mildew, and nutritional differences as exhibited by leaf scorch symptoms.

All these characteristics go to make up " rootstock influence," which cannot be detected merely by measuring the growths of one or two-year-old trees. When such trees on clonal roots vary in size, they still behave similarly in all other aspects of " stock effect." Although no single term such as " vigorous " or " semi-dwarfing " is adequate, vegetative stocks have fallen readily into groups with certain common characteristics. Examples of the practical value to fruit growers are given.

There is evidence to show that part of this influence is in the stem of the rootstock, the path by which the materials, collected by the roots, pass to the scion. This has been proved by the use of pieces of stock stems used as " intermediates " between roots and ultimate scion. Workers in America studying the effect of scions grafted direct upon seedling roots concluded that stock influence lay in the stem portion.

Evidence is now presented for the first time which suggests that stock influence is also significantly apparent upon two varieties of scion worked directly upon clonal vegetative piece roots in two repeated experiments, the trees being three and four years old respectively. The influence is apparent in type and duration and, in some cases, actual length of growth, early fruit bud formation, percentage of blossom setting, amount and quality of fruit, and in maiden growth and weight of these trees lifted at two years old.

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PLATE I.

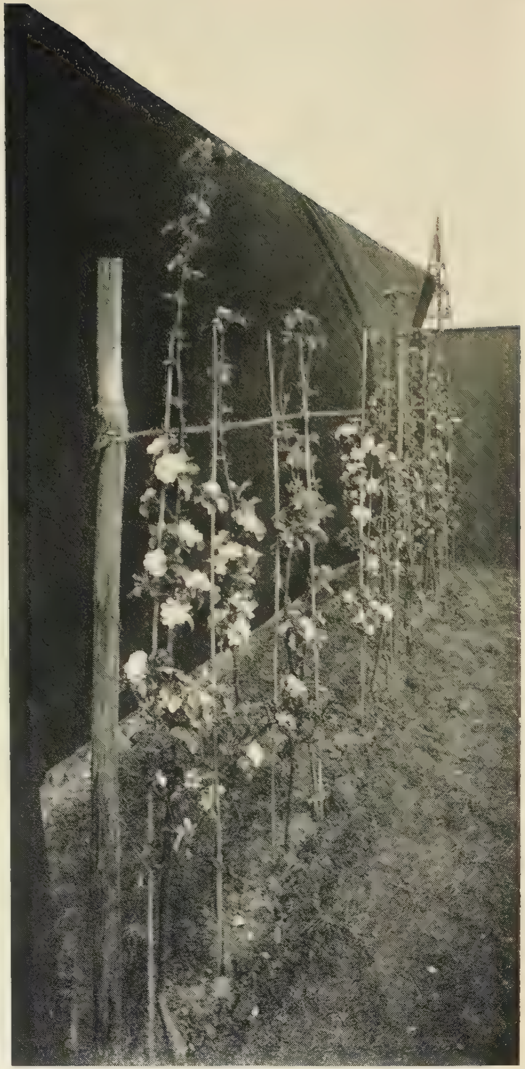

FIG. 1.

Three year Beauty of Bath on No. VIII piece roots. Note dwarfed growth and numerous blossom buds on one, two and three year old wood.

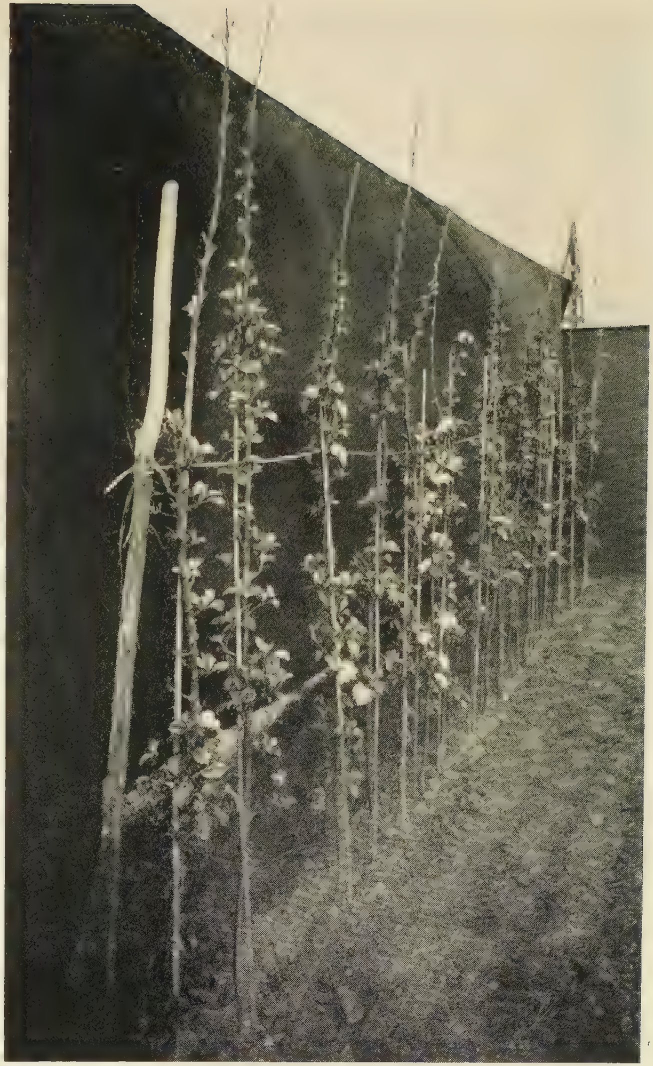

FIG. 2.

Compare three year Beauty of Bath on No. VI piece roots. Vigorous growth and very few blossom buds.

PLATE II.

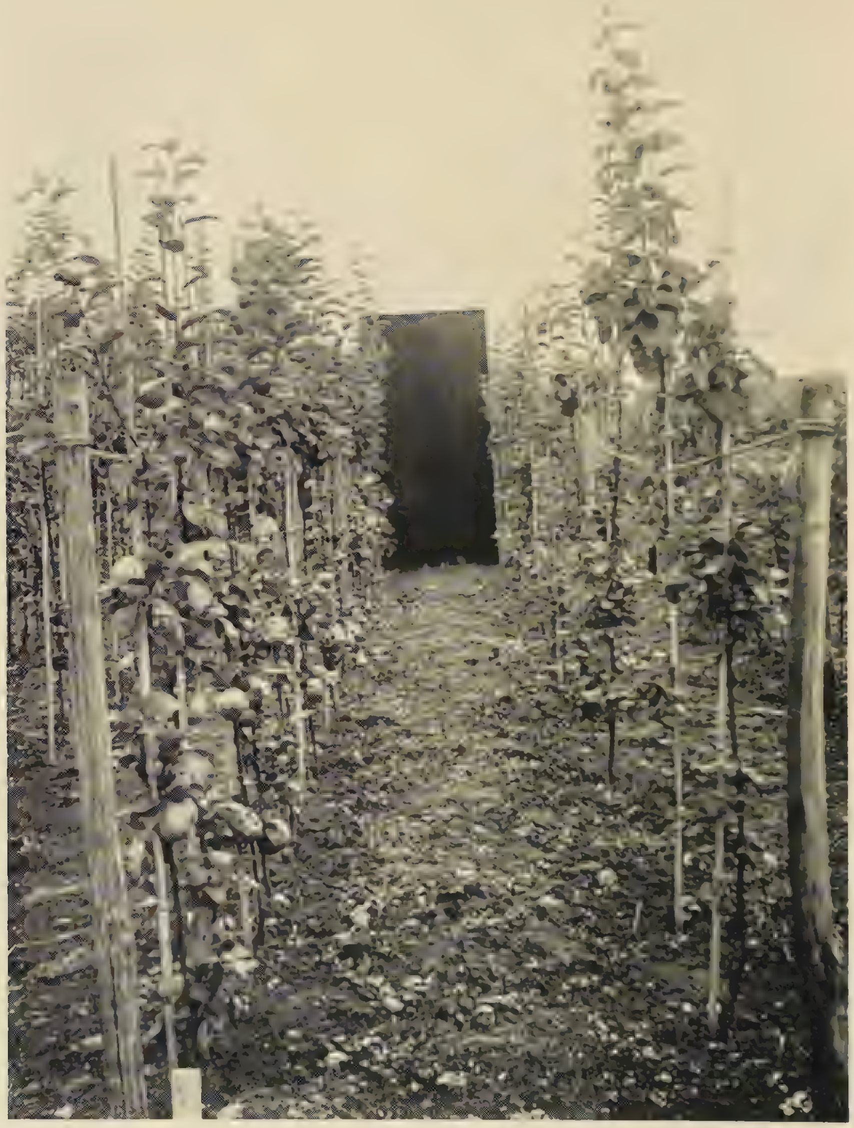

FIG. 3.

Two year Grenadiers in nursery, grafted on piece roots of No. IX (left) and No. VI (right) vegetatively raised rootstocks. Note early cropping on No. IX.

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THE MORPHOLOGY AND CYTOLOGY OF THE APPLE FRUIT, WITH SPECIAL REFERENCE TO THE BRAMLEY'S SEEDLING VARIETY.

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In a previous paper (11), the stages of the external development of the fruit (i.e., bud, flower and setting of the fruit) were correlated as far as possible with the cytological development recorded by the microscopic examination of fixed material. Observations of a general character were also made on the epidermal structure of a few varieties of apple.

In the present communication, the results of a more extensive morphological and cytological study of the developing apple fruit are presented. Records were made of (1) the average weight of a sample of apples throughout the season ; (2) the increase in cell-size ; (3) the increase in the thickness of the cuticle. These were correlated with the weather records for the season. In this way, it was hoped that data would be obtained which would show the effect of the growing season on the behaviour of apples in storage.

These morphological and cytological records were made on developing apples on the tree, and on mature apples in storage. In addition, the lenticel structure was examined to determine the range of structure present in a single variety. The cytological observations included those on the structure of the cell-wall throughout the season, together with observations on the nucleus and cytoplasm. Starch formation and its disappearance were studied in some detail to provide data as to the exact formation of the starch grain in the apple and the stages in its disappearance. The distribution of the chloroplasts and the amount of starch formation taking place in them was also investigated.

Observations on mature apples in storage included measurements of cuticle thickness and the structure of the " eye " of the apple. The latter was investigated in the hopes that it might throw light on the method of invasion of the fungi causing " eye-rot " of apples.

MATERIAL AND METHODS.

The material for these investigations was gathered from the same tree at Histon, near Cambridge, at intervals of about ten days from June 2nd to November 18th, 1930. It was examined in a fresh condition, and samples were preserved in formalin acetic alcohol, Bouin's solution or in 50 per cent. alcohol. Some of this preserved material was embedded in wax with a melting point of 55° C. in the usual way and microtomed sections were prepared. When the apples were comparatively large and had correspondingly large intercellular spaces, it was found that the cell-walls always collapsed slightly during the embedding, but sections of fresh material were always used for comparison with the microtomed preparations. The sections of the epidermal part of the apple were stained with Sudan III in glycerine and alcohol and were mounted in glycerine jelly. Those of the " eye " of the apple were stained with Cotton Blue in lacto-phenol according to the method worked out by Hough (8) for microtomed preparations. Various stains were found useful for the examination of the cell contents of the flesh of the apple. Thionin Blue and Orange G., Gentian Violet, Brazalin, and Safranin and Picric Aniline Blue were all used.

THE DEVELOPING APPLE ON THE TREE.

(*a*) *The General Development of Bramley's Seedling.*

Comparatively little is known about the relationship, if any, which exists between the weather conditions experienced by a crop of apples and the behaviour of those apples in storage. The results of a series of experiments done at this Research Station (12) indicated, however, that good keeping qualities were associated with warm, dry weather during the few weeks before they were gathered. Horne (7) showed that the parasitic virulence of some fungi which attack apples varied in different storage seasons according to the weather experienced during the growing season. For example, Cox's Orange Pippin apples picked in 1921 and stored at 1° C. after inoculation were all rotten in ninety days. In 1922, very little effect was made by the fungus 143 days after inoculation. 1921 was a hot, dry season while 1922 was a wet season and the apples ripened late. Such results indicate that the various types of season experienced by the English apple crops may bring about morphological and physiological variations which influence the storage life of the fruit.

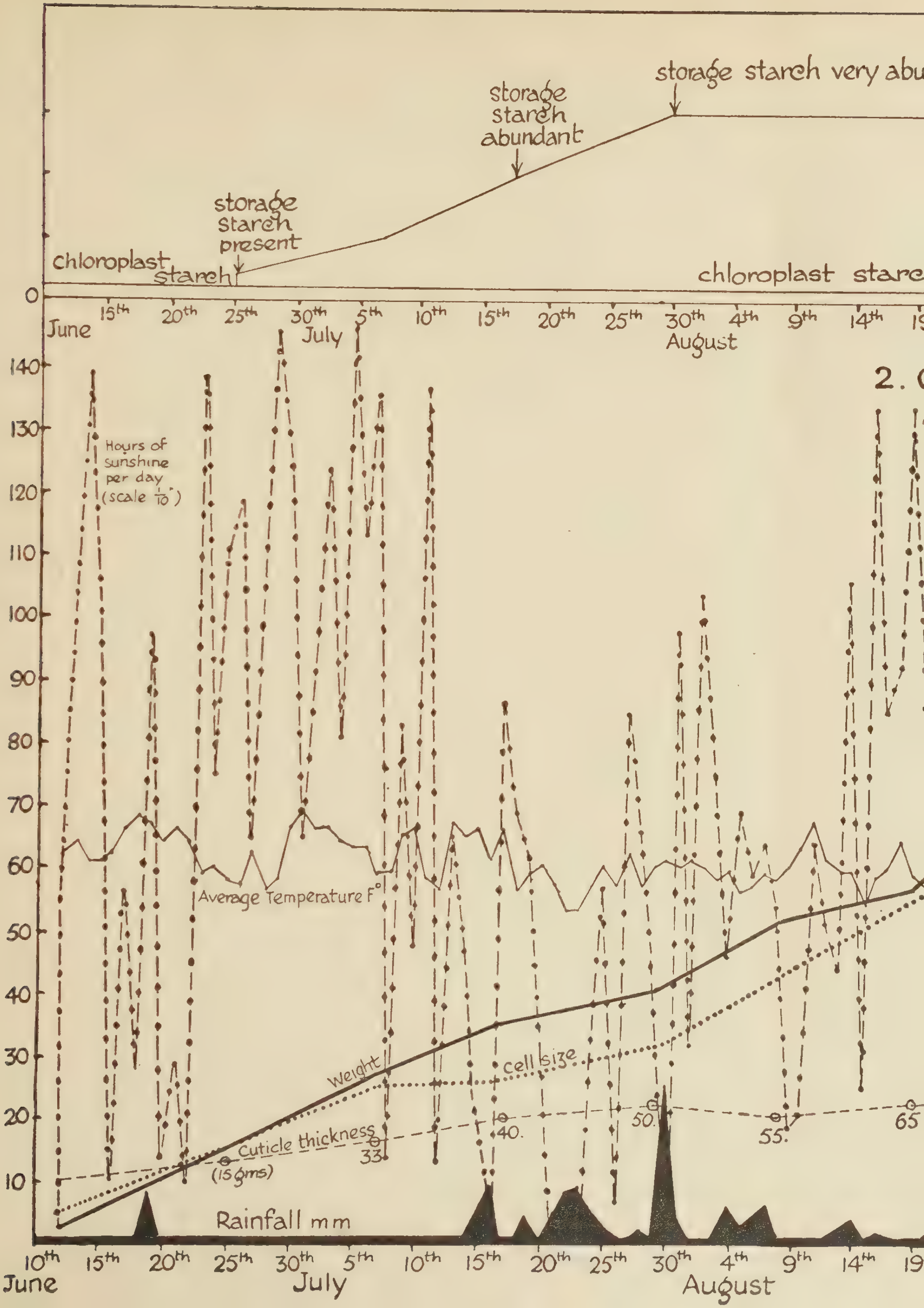
During the season 1930, the following records were kept: (1) the average weight of a sample of forty to fifty Bramley's Seedling apples which were picked every ten days from June 12th until November 18th, 1930. (2) The average size of the cells of the flesh of the apple. This was determined from camera lucida drawings of sections of fresh material which were made on squared paper. Since the shape of the cells of a developing apple is by no means uniform, the average area of the cell in its widest cross section was determined. This method gave a general estimate of the actual increase in size of the cell and it was sufficiently accurate to show any rapid change in size during the course of the season. (3) The amount of cuticle deposited on unit length of epidermis. This was also measured by means of camera lucida drawings on squared paper. This method gave more satisfactory results than a measurement of the skin thickness only. The latter takes no consideration of the amount of cuticle deposited on the radial walls of the epidermal cells, which varies very much in different varieties of apples under different conditions. The unit length of epidermis was taken as 26 mm. on the squared paper, and about fifty determinations were worked out for each picking of apples. When the apples had started to colour, the difference in the amount of cuticle on the red and on the green side of the apple was determined and also the difference in the amount deposited on the small and the large apples. (4) The starch content was determined by eye. The figures used in the graph in Fig. 1 are merely arbitrary and show the general trends of starch accumulation and disappearance throughout the season.

The results obtained from these determinations will be seen in the accompanying graph (Fig. 1), together with the records of temperature, sunshine and rainfall. The weather during the summer of 1930 was characterized by only two periods of bright sunshine, i.e., from June 22nd to July 11th and from August 15th to September 5th. During the first period practically no rain fell, but during the second period a considerable amount fell at intervals. The apples left on the tree until November 18th experienced some very cold weather during the last fortnight on the tree. The average daily temperature varied very little between 55° F. and 70° F. from June 12th until the end of September, except for a short, very hot spell from August 26th to 30th.

The average weight of the apples increased steadily until the end of September, but the rate of increase fell off during two periods of very bad, sunless weather. A further slight increase in the average weight was obtained on October 28th and November 10th, but probably the weight of the apples on the tree remained constant or only increased slightly during October and November. The apples had reached approximately the maximum weight at the normal time of picking in this part of the country.

The maximum cell-size was attained about a month before the maximum weight had been reached. The rate of increase was approximately constant except for a falling off when the heavy accumulation of starch in the cells was beginning in the middle of July. This falling off in the rate coincided with the early part of the first long spell of bad weather.

The amount of cuticle deposited on the epidermis showed a steady increase until the end of October. The later determinations varied from apple to apple and according to whether the red or the green side of the apple was used. The amount of cuticle deposited on the red side of the apple was less than that on the green until finally the apples were coloured more evenly all over. For example, on November 12th the amount of cuticle in apples of very different sizes (130 gm., 94 gm. and 57 gm.) was practically the same in each case. Similar differences in the amount of cuticle deposited on the red and green sides of apples were noted by Markley and Sando (9) in some American varieties


storage starch very abu
storage starch abundant
storage starch present
chloroplast starch
chloroplast starc
0
June
15th
20th
25th
30th
July
5th
10th
15th
20th
25th
30th
August
4th
9th
14th
19
2. (
140
130
120
110
100
90
80
70
60
50
40
30
20
10
Hours of sunshine per day (scale 1/10)
Average Temperature F°
Weight
cell size
Cuticle thickness
(15 gms)
33
40.
50.
55.
65
Rainfall mm
10th June
15th
20th
25th
30th
5th July
10th
15th
20th
25th
30th
4th August
9th
14th
19

of apples ; they used chemical methods of determination. Presumably, the deposition of cuticle continued for a longer period on the sunny side of the apple than on the shady side. Some of the small apples in the late part of the season had a greater cuticle deposit than the larger ones. It appears that from the above results the rate of increase in the amount of cuticle throughout the season is not affected by the weather conditions.

The starch present in young apples to the end of June consisted solely of chloroplast starch. After that time, storage starch began to appear and the amount increased steadily until the end of July. Starch was very abundant in the cells during the whole of August, but after that, the amount gradually decreased until the cells were practically starch free by the end of October.

The growth in length of a Bramley's Seedling apple takes place almost entirely during the eight weeks after the fruit has set. Sample fruits were cut in half and measurements were made of the length from the rim of the calyx cup to the top of the stalk. In the sample of mature Bramley's Seedling studied, this measurement varied from 2.8 cm. to 3.0 cm. and this length was reached by the middle of July. The zone of rapid growth in an apple (i.e., the region of the development of the flesh) is confined to a relatively small zone between the point where the vascular bundle from the stalk begins to branch and the base of the style. From an anatomical point of view, it is interesting to find that no endodermis or similar restricting layer which ceases just below the base of the apple is present. The excessive development of the vascular supply at the base of the apple may play a part in determining this narrow zone of growth. The rapid increase in the extent of the flesh after the apple has set results in the concentration of the vascular tissue at the base of the carpel cavities, followed by the burying of the top part of the stalk in the surrounding flesh. These stages of growth are illustrated in Fig. 2.

The burying of the stalk takes place to a much greater extent in apples developed in a terminal position in the cluster than in those developed laterally. This fact was noted by Hinton and Swarbrick (6) in Allington Pippin, Newton Wonder and King Edward VII. Growth at the base of the apple continues later and is more pronounced on one side than on the other, producing a kind of short beak down the stalk. These authors found, too, that the lateral fruits were much sweeter than the terminal ones, and were the better fruit from the consumer's point of view. In Bramley's Seedling, this difference in shape in the apples developing in the two positions is also apparent. The late phase of growth in this apple takes place below the place where the vascular supply branches at the base of the apple. A single branch bundle is given off from the main vascular supply just below the point where the carpellary supply branches off. The renewed growth evidently takes place between these two regions. The side on which the extra growth takes place is that supplied by the

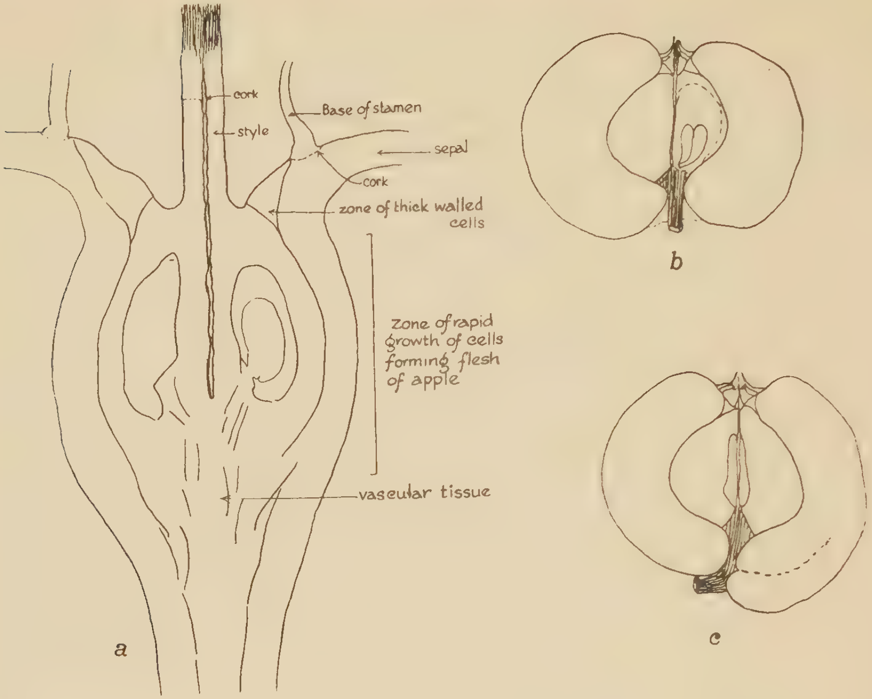

cork
style
Base of stamen
sepal
cork
zone of thick walled cells
zone of rapid growth of cells forming flesh of apple
vascular tissue
a
b
c

FIG. 2.

a.—Longitudinal section of apple after setting (x6).
b.—Longitudinal section of lateral fruit of Bramley's Seedling Apple (half natural size).
c.—Longitudinal section of terminal fruit of Bramley's Seedling Apple (half natural size).

additional branch vascular bundle. This additional bundle was not present in the lateral fruits examined. In 1931, these trees carried a very small crop indeed and the majority of the apples were terminal fruits. It seems, therefore, that the terminal flowers are more likely to set under adverse fruiting conditions than the lateral flowers.

(*b*) *Morphological Development of the Skin of Bramley's Seedling.*

1. EPIDERMIS.

In the apples picked on June 12th the epidermal cells were still brick shaped and were considerably deeper than they were wide. The average depth of the cells was 43μ and the width varied from 11μ to 21μ according to whether the cells had divided recently or not. The epidermal cells were still undergoing divisions at this date (June 12th), but these ceased about this time and no signs of any recent divisions of the epidermal cells were observed in sections of apples picked on June 20th.

Measurements of the epidermal cells showed that during June, July and August they were gradually being stretched in a tangential direction. Part of this stretching was due presumably to the pressure of the developing cells of the flesh tissue, which were increasing gradually in size until the end of August. The walls of the epidermal cells evidently remain plastic for a long period of time. It is possible that large quantities of oily and fatty materials pass through and in contact with the walls of the epidermal cells, which may help to keep the walls plastic.

Measurement of Epidermal Cells in a Radial and Tangential Direction.

Date.	Radial.	Tangential.
12/6/30	43μ	11μ
	43μ	21μ
25/6/30	28μ	43μ
	25μ	28μ
	21μ	28μ
	21μ	21μ
7/7/30 to 20/9/30	21μ	28μ
	21μ	36μ
	14μ	43μ
	21μ	51μ
8/10/30 to 21/10/30	15μ	43μ
	14μ	40μ
	17μ	60μ
	11μ	23μ
	9μ	37μ
	11μ	47μ
	14μ	43μ

The epidermal cells and the cuticle of Bramley's Seedling apples form a very even layer over the outside of the apple. The continuity of the cuticle is interrupted by stomata and the bases of hairs which break off in the early stages of development of the apple. The former may disappear owing to their transformation into lenticels or they may still be present in mature apples. The walls of the guard cells of the old stomata become thickened and the sub-stomatal cavity is more or less blocked by cells with suberized walls (Fig. 3 a, b).

The cuticle increases gradually in amount throughout the growing season. The amount varies somewhat from apple to apple owing to the extension of the cuticle down the radial walls of the epidermal cells. In some apples, the epidermal cells, instead of being oblong in cross section, are more or less oval in shape owing to the breadth of the deposit between the radial walls of the neighbouring cells.

The increase in the amount of cuticle deposited between the radial walls of the epidermal cells increases the surface area of the epidermis without the formation of cork ; this may be found to be typical of apples with comparatively small numbers of lenticels such as the Bramley's Seedling (Fig. 3 c, d).

Some interesting variations in the deposition of the cuticle were noted in the mature apples. Several apples showed depressions in the cuticle which were bridged over by a layer of cutin in the early part of the season. These " bridges " break with the continued pressure on the epidermal cells which tends to straighten out the depression (Fig. 4). Such variations show possible methods of adjustment between the extrenal layer and the internal layers of Bramley's Seedling, without the formation of cork.

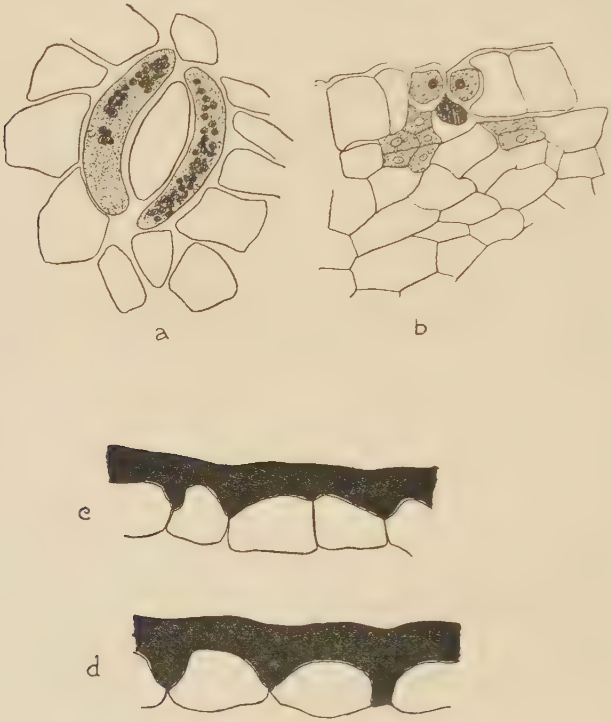


FIG. 3 (x350).

(*a*) Stoma of mature apple in surface view.

(*b*) Stoma in section, showing blocking of substomatal cavity.

(*c*) Transverse section through epidermal cells 1/9/30.

(*d*) Transverse section through epidermal cells 20/9/30 showing difference in amount of cuticle deposited on radial walls.

Fatty deposits between the lower tangential wall of the epidermal cells and the sub-epidermal cells were most frequently found in the smaller apples which were left on the tree until the middle of November and also in samples of apples off trees which had been starved of potassium. Fat metabolism evidently continues while the apple is on the tree up to a comparatively late stage, but changes may occur in some apples in the walls of the epidermal cells which prevent the deposition of fatty substances on the outer tangential wall of the epidermis and the sub-epidermal cells (see Fig. 4, c).

2. THE LENTICELS.

It has not been possible to analyse the factors responsible for lenticel formation. The variation in the number of lenticels is large in different varieties of apples, but the variation is less in individual apples of the same variety. The amount of pressure on the hair bases and stomata presumably varies in different apples and this will be influenced by the plasticity of the epidermal cells and in the later stages by the amount of separation of the original radial walls of the epidermal cells.

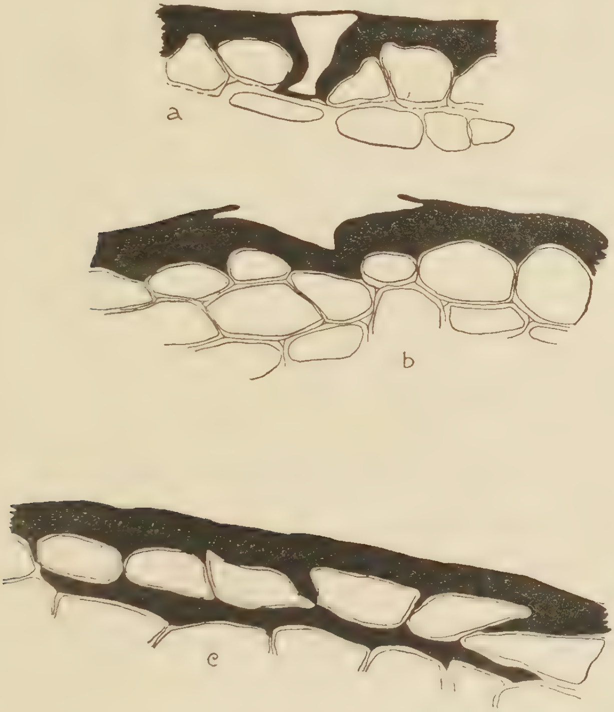


FIG. 4 (x350).

a and b.—Transverse section through epidermal cells showing depressions in cuticle bridged over, and later with " bridge " broken.

c.—Transverse section through epidermal cells showing fatty deposits in lower tangential walls of epidermal cells.

In a previous section of this paper it has been mentioned that stomata which no longer function may be present in mature apples. Generally, however, the stomata become unrecognizable when the apple is in an early stage of development owing to their transformation into lenticels. Before this takes place, the walls of the guard cells become thickened and the sub-stomatal cavity may become plugged with a brown substance. The walls of the cells surrounding the sub-stomatal cavity become suberized and later these cells also become filled with a brown deposit, and cork formation occurs which cuts off the dead cells from the cells of the living flesh below. Microchemical tests suggest that this brown substance is some form of tannin.

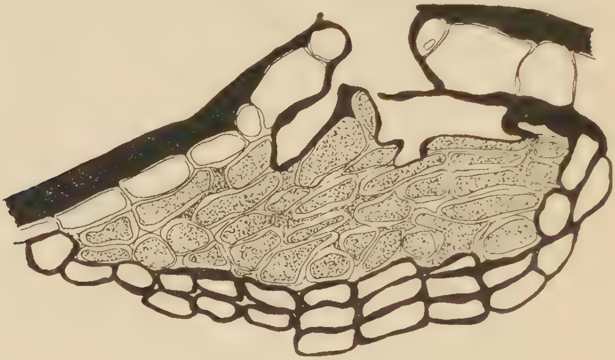


FIG. 5.

Section through a lenticel derived from a stoma (x350).
(Walls in solid black are those which stain with Sudan III.)

In the majority of mature lenticels it is impossible to tell whether it has been derived from a stoma or not, as the guard cells are usually unrecognizable at that stage.

Some of the lenticels of a mature Bramley's Seedling appear as irregular white dots on the surface of the apple with or without a brown spot in the centre ; others form a minute brown patch on the surface of the skin which varies considerably in size and shape from apple to apple. The first type of lenticel is formed by the enlarged and thickened bases of the hairs ; these can apparently undergo a considerable amount of stretching without any splitting. The walls of the sub-epidermal cells below these hair bases are suberized in one or two layers of the cells, but very little cork formation occurs. Type 2 (white dots with a brown centre) are formed by the hair bases which have enlarged more than those of type 1. Below the lenticels of type 2 the suberized cells have loosened and are being forced up through the base of the hair. Cork formation is much more extensive in this type than it is in type 1.

Lenticels which appear as brown marks on the surface of the apple are those formed in connection with the hair bases which have split. These lenticels may be very shallow and form hardly any depression on the surface of the

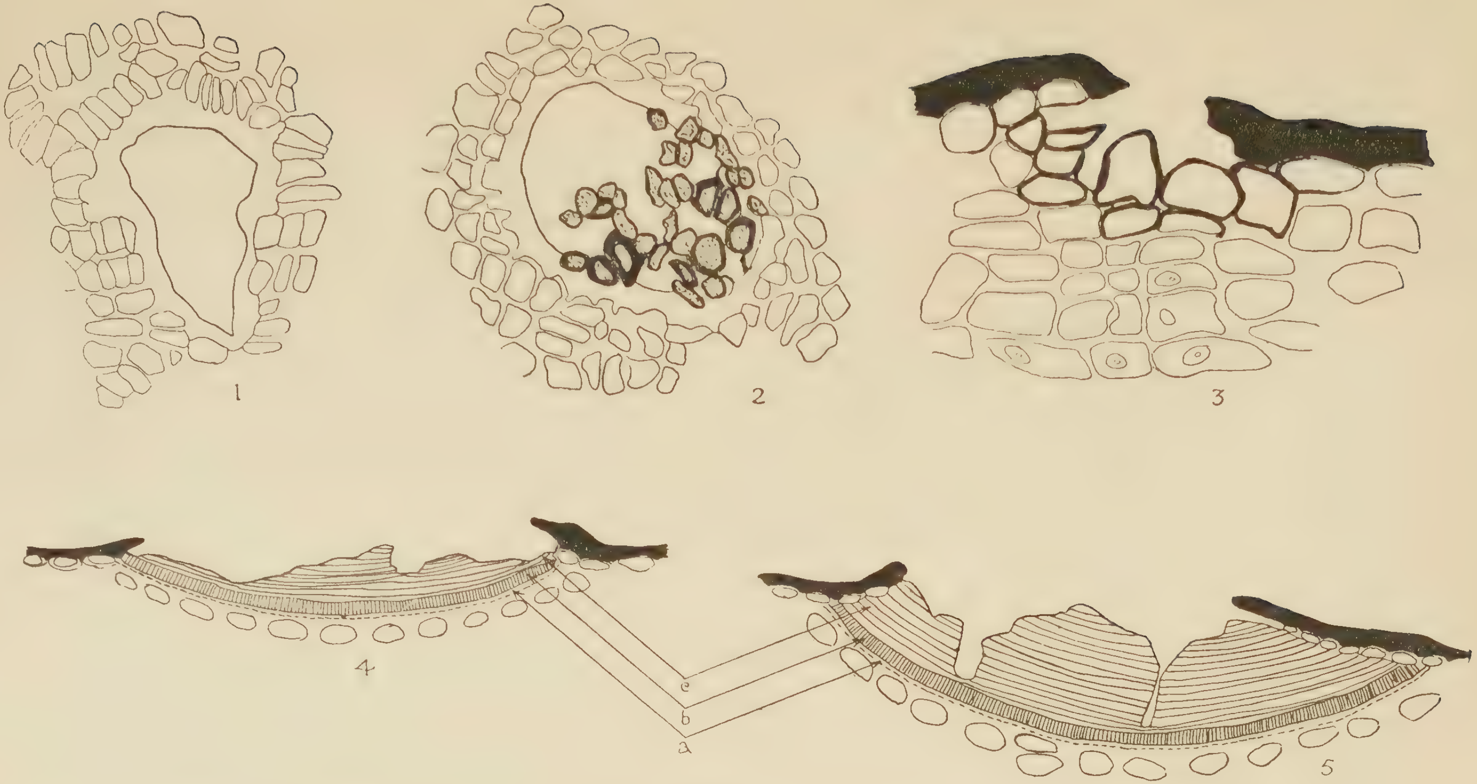


FIG. 6.

1.—Surface view of hair base without brown centre (Type 1)
2.—Surface view of hair base with brown centre (Type 2)
3.—Transverse section of hair base (Type 1). Solid black represents walls stained with Sudan III
(x230).

4.—Diagram of shallow type of lenticel (Bramley's Seedling)
5.—Diagram of deep type of lenticel (Ecklinville)
a.—cork meristerm
b.—cork
c.—dead brown cells
(x100).

apple ; others are deep and cork formation has occurred at a considerable distance below the surface. Generally in the latter there are many cracks between the suberized cells, and such a type of lenticel probably represents a favourable habitat for the germination of fungal spores. Fungal hyphæ were frequently found in these lenticels and were nearly always absent in the shallow type of lenticel. In the season of 1930, the shallow lenticels were of far more general occurrence than the deep type, and they are the characteristic type of lenticel formed in Bramley's Seedling.

The amount of variation that may occur in the lenticels of different varieties of apples were seen when the lenticel structure of the variety Ecklinville was compared with that of Bramley's Seedling. The tree from which the Ecklinville apples were picked was adjacent to the Bramley's Seedling tree used for this work. The lenticels of Ecklinville were very prominent and became brown at a comparatively early stage. They were all formed under hair bases which had split considerably and the edges of which formed a more or less loose brown flap of tissue. A large amount of cracking had taken place in the dead cells below the hair base and cork had been formed some way below these cells (Fig. 6, 5). The extensive growth of fungal hyphæ in these lenticels of Ecklinville was very noticeable since the lenticels of Bramley's Seedling were almost free from hyphæ and both varieties were subjected to the same spraying treatment. The deep type of lenticel is evidently one favourable to the growth of fungal spores. When apples possessing this type of lenticel are used for storage purposes, they go into storage with a large fungal population already present in the lenticels.

(*c*) *Cytological Development of Bramley's Seedling.*

1. The flesh of the apple fruit is made up of a loose tissue of cells, each of which consists of a cellulose wall (cellulose is a sugar-like substance and is usually the main constituent of the cell-walls of plants) surrounding the living protoplast, which here forms a lining layer to the wall. The living protoplast consists of a clear, somewhat granular substance (the cytoplasm), in which is embedded a denser oval body—the nucleus (Text, Fig. 7, b). The walls of the cells which touch each other are held together by the deposition of a pectic substance which " cements " the cells together and forms the middle lamella. This part of the cell-wall stains with ruthenium red. The tissue forming the flesh of the apple is characterized by the presence of large air-spaces between the cells ; these increase in size gradually throughout the season as the cells enlarge and come apart from each other owing to the softening of the middle lamella.

In a previous section of this paper, figures have been given to show the increase in size of the cells of Bramley's Seedling during the season. This increase took place from June to the middle of September and represented an increase of from seven to ten times in the size of the cells of an apple which had set. Comparatively little decrease in thickness of the wall was visible while this rapid increase in size was taking place ; the individual cells gradually came apart and so formed the large and irregular intercellular spaces characteristic of the mature flesh of the apple.

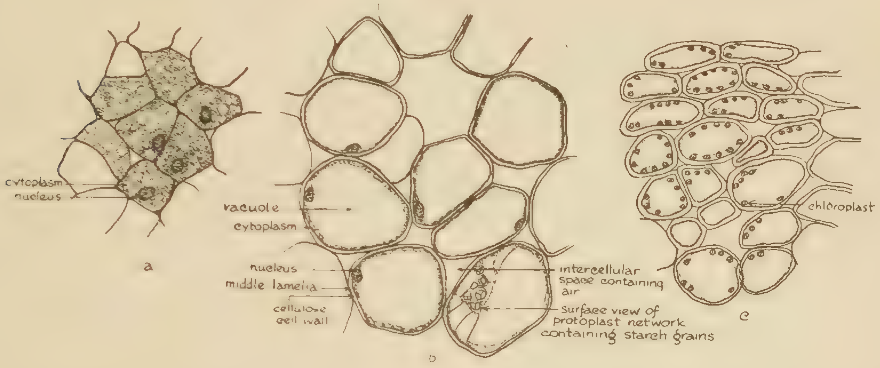

cytoplasm
nucleus
a
vacuole
cytoplasm
nucleus
middle lamella
cellulose cell wall
intercellular space containing air
surface view of protoplast network containing starch grains
b
chloroplast
c

FIG. 7.

a.—Section of cells of the flesh of apples of the meristematic stage—showing thin cell-walls and cytoplasm filling cell.

b.—Section of cells of the flesh of apples when mature—showing separation of the cells and cytoplasm forming lining layer to wall.

c.—Section of sub-epidermal cells of apple showing chloroplasts. (Drawings slightly diagrammatic.)

It is difficult to believe that cellulose synthesis is not taking place during the rapid increase in size of the cells, otherwise there would be a very noticeable decrease in the thickness of the cell-walls during this time. Haynes and Archbold (5) consider that most of the cellulose and pectin are laid down before the synthesis of starch begins. Assuming this, they use the variation in the amount of alcohol insoluble material as an indication of the amount of starch formed, since the amount of cellulose and pectin is held to be constant after the apple has set. They point out, however, that such an approximation is not altogether satisfactory, because a considerable increase in the alcohol insoluble residue is obtained before any storage starch is present in the apple. Judging by microscopic observation, some of this increase in the alcohol insoluble material is due to the synthesis of cell-wall materials. When more accurate chemical methods have been devised for the estimation of starch, it will probably be found that the synthesis of cell-wall material continues to a varying extent for some time during the growing season.

During the separation of the cells, there were no indications of the pectic changes observed by Carré and Horne (2 and 3). The presence of the pit-like areas mentioned by the writer in her previous paper on this subject (11) was again noted. In the early stages, the staining of the cell-walls was uniform and the distinction between it and the middle lamella was not noticeable until the cells had begun to come apart rapidly. The middle lamella was then in a condition when it would pick up any stain readily. The pectic substances tended to remain round the pits in the cell-walls after the cells had separated. When the cells had reached their " equilibrium point " and no relative readjustment of the cells are going on in the flesh, these pectin deposits probably dry round the pits. The latter evidently represent areas capable of holding the cells together and of checking the separation when the softening of the middle lamella is taking place.

Some interesting results were obtained from a series of maceration experiments* which were carried out during the growing season. The following solutions were used: hydrochloric acid, oxalic acid, and potassium hydrate in 5 per cent. aqueous solutions and Eau de Javelle. The Sections of the flesh were placed in the reagent for twenty minutes in a corked tube on a glass slide on the top of the embedding oven which kept the solutions at a constant temperature of about 44° C. After the action of the first reagent the sections were washed quickly in distilled water and were left in this for five minutes before they were placed in the second reagent. Apples picked not later than 29.7.30 showed no, or only very slight maceration with hydrochloric acid or with oxalic acid followed by potassium hydrate. Maceration occurred only if Eau de Javelle (i.e., potassium hypochlorite, used to dissolve out protein substances) was used before hydrochloric acid, followed by potassium hydrate.

Date of Picking.	(1) HCl + KOH	(2) Oxalic acid + KOH	(3) Eau de Javelle + HCl + KOH
7/7/30 ..	No maceration (i.e. middle lamella still present). Walls stain with Ruthenium red.	No maceration. Walls stain with Ruthenium red.	Slight tendency for cells to come apart after Eau de Javelle + HC1, maceration complete after KOH added.
17/7/30 ..	No maceration. Definite wall stain with Ruthenium red.	No maceration.	Maceration, i.e. middle lamella dissolved.
29/7/30 ..	Very slight maceration. Walls stain with Ruthenium red.	No maceration unless Eau de Javelle used first.	Maceration.
8/8/30 ..	Maceration but walls stain with Ruthenium red.	Maceration, no stain in walls with Ruthenium red.	Maceration more rapid than in 1 and 2.
1/9/30 ..	Maceration.	Maceration.	Maceration.

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* Maceration experiments are those in which the purpose of the experiment is to bring about the dissolution of the middle lamella (and hence the separation of the cells) by chemical means.

The cells of apples picked not later than 8.8.30 would macerate to a certain extent when hydrochloric acid or oxalic acid was followed by potassium hydrate. Maceration was much more extensive and more rapid when Eau de Javelle preceded the use of hydrochloric acid and potassium hydrate. From 1.9.30 any of these reagents would bring about maceration, as is shown in the accompanying table.

These results suggest that the middle lamella is changing slowly during the growth of the apple on the tree. It is evidently losing some substance—possibly of a protein nature—which resists macerating reagents and other substances which tend to bring about the breakdown of the middle lamella. The separation of the cells was a very gradual process and presumably was due to the rapid increase in the size of the cells acting on a middle lamella which was becoming more plastic and gelatinous.

2. NUCLEUS AND CYTOPLASM.

Archbold (1) has already shown that nitrogenous substances, principally in the form of proteins, are accumulated during the earliest stages of the development of the apple. It is not surprising, therefore, to find that cell-division ceases at an early stage—about the beginning of June—and also that the size of the nucleus shows no increase in size after the end of June.

After the earliest meristematic stages (i.e., when cell-division is going on and the cells are completely filled with protoplasm), the cytoplasm remains in close contact with the wall of the enlarging cell and eventually becomes very attenuated in a more or less net-like way, and it is in the meshes of this network that the starch grains are formed (see below). The protoplast comes away from the cell-walls with difficulty when sections are placed in hypertonic solutions of cane sugar or common salt. This may be due to cytoplasmic connections across the cell-walls, the actual presence of which have not yet been demonstrated. Some plasmolysis* experiments demonstrate the close contact between the protoplast and the cell-wall. Practically no plasmolysis occurs when sections are placed in hypertonic solutions of cane sugar until the sections are pumped free from air. The air-filled inter-cellular spaces evidently act as an efficient barrier to the actions of external solutions on the protoplast.

Plasmolysis was watched in a large number of sections and it was very noticeable how the first part of the protoplast, and sometimes the only part, to come away from the wall was always that opposite an intercellular space. The protoplast came away comparatively rapidly between the pits, but was held at these points for some considerable time. The connection between the wall and the protoplast is firmer where the pits of neighbouring walls face each other and it is reasonable to think that this may be due to the presence of protoplasmic connections across the pit. A similar sequence of events was noted when the cells forming the inner skin of onion scales were used for plasmolysis. The pits in these cell-walls were larger than those of apple and protoplasmic connections across the pits could be demonstrated.

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* Plasmolysis—the withdrawal of the cytoplasm from the cell-wall where the cell is surrounded by a solution which withdraws water from the vacuole (i.e., a hypertonic solution).

3. THE CHLOROPLASTS.

The majority of the chloroplasts in the Bramley's Seedling apple are to be found in the sub-epidermal cells, but they are also scattered through the cells of the outer layers of the flesh. In the youngest apples the sub-epidermal cells have only slightly thicker walls than the cells of the flesh within and are scarcely distinguishable from them. As soon as the apple has set, however, the sub-epidermis soon becomes distinguished from the flesh owing to a rapid increase in the thickness of the walls of the component cells and the elongation of these cells in a tangential direction. The chloroplasts are very abundant in these cells and they are often so closely packed that it is impossible to distinguish the nucleus.

Starch does not accumulate in the chloroplasts of the Bramley's Seedling to any great extent, for only very minute grains were found in the chloroplasts. It is suggested that the sub-epidermal cells may be the seat of fat metabolism and that the carbohydrates formed during photosynthesis are transformed into fatty substances which pass out of the cell and are finally deposited on the cuticle. When these cells were stained with Sudan III in glycerine and alcohol the lining layer of protoplasm always showed the presence of minute red fatty globules.

Chloroplasts are present in the apples throughout their normal storage life. They remain green for a considerable time and only lose this colour when the apple has turned a very yellow colour.

4. STARCH STORAGE IN BRAMLEY'S SEEDLING.

In the season 1930, storage starch (i.e., starch deposited in the cytoplasm and not in chloroplasts) in Bramley's Seedling did not appear in the cells until the end of June, and it gradually increased in amount until the end of July. From this time until the beginning of September, abundant starch was present in the cells and from then onwards a definite decrease in the amount was noticeable. The decrease continued while the apple was ripening and when the apple went into storage very little starch remained.

The method of starch formation is of interest in apples because the grains do not appear to be deposited in the usual type of leucoplasts. The network arrangement of the mature protoplast has been mentioned in a previous section of this paper, and also the fact that the starch grains are deposited in this network. The formation of the starch grains in apples resembles in appearance the formation of aleurone grains from the contents of vacuoles rich in protein substances, except that in apples the " vacuoles " in which the starch is deposited are more definitely delimited (Text, Fig. 7, b).

The cytoplasm forming the network of the protoplasm condenses to form a honeycombed structure with definite walls separating the cavities. These cavities are quite distinct for some time before the starch grains are deposited and they enlarge somewhat before this takes place. When the storage of starch begins, either a single grain or two or three smaller ones are deposited in each cavity. This starch-forming network seems to be a definite part of the protoplast for it persists after the disappearance of the starch grains throughout the life of the protoplast.

In this connection, it is interesting to note that Schimper in 1880 used the term " Stärkebildner " for the cell-inclusions in which starch was formed (10). He divided them into these classes according to their shape : (1) "Stärkebildner," which were round ; (2) those which were spindle shaped ; (3) those which were round at first, but later became elongated. In the drawings of these starch-forming bodies, the starch grains are formed externally except in some cases in which innumerable minute grains are shown which completely fill the cavity of the " leucoplast " or else a single starch grain or crystalloid is shown in the widest part of the elongated leucoplast.

In the apple, starch formation seems to be so much more of the nature of a deposition of the grain within a modified protoplasmic network than a deposition within isolated bodies in the cell. The latter are said to be temporary bodies, while the former persist throughout the life of the protoplast. These differences may be bound up with the great rapidity of starch formation in the apple and also with a high rate of metabolism and the concentration of the substances out of which the starch grains are formed.

THE MATURE APPLE IN STORAGE.

(*a*) *Cytological changes in Stored Apples.*

When a Bramley's Seedling apple goes into storage under normal conditions, it has generally reached its maximum weight, although it may increase slightly in weight in favourable seasons if left on the tree. The apple at this stage generally contains a small quantity of starch which, however, soon disappears and, at the same time, the acidity of the apple is decreasing.

After gathering, the morphological and cytological changes going on in a Bramley's Seedling are not very striking. The nucleus remains the same size throughout the storage life, and changes in the cell-wall and middle lamella appear to occur very gradually. The latter changes result in an increased separation of the individual cells of the flesh, which is characteristic of apples which have become mealy. Some varieties of apples are more liable to become mealy under storage conditions than others. In Bramley's Seedling, this tendency is not very marked. Carré and Horne (3) found that the breakdown of the middle lamella under storage conditions began with a swelling of the pectic substances at the air-space angles, followed by the deposition of aggregates of pectic material on the separated walls. The so-called " crescents and discs " of these authors also underwent changes. The "crescents" became surrounded by diffuse stainable material which coalesced to form a continuous band. They gradually lost stainable material and disappeared. The " discs " became swollen and also finally disappeared.

After the growing season of 1930, middle lamella breakdown was very gradual and did not take place to any marked degree. The entire middle lamella at first tended to become more heavily stained, suggesting that it was becoming more gelatinous. Any subsequent separation of the cells did not leave behind any aggregations of pectin on the wall. After several months' storage, the tendency was for the apple to shrivel and the middle lamella to shrink, doubtless owing to the loss of water during storage. Apples suffering from low temperature internal breakdown were the only ones to show aggregations of pectin on the walls, which were obviously derived from the breakdown of the middle lamella.

The elaborate changes in the cell-wall pectin (i.e., crescents and discs) mentioned by Carré and Horne were also not observed during the storage season 1930 to 1931. Any changes going on in the walls were very gradual and the only part of the wall which stained definitely with Ruthenium Red was the pectin material deposited round the pits in the walls. Judging by the staining reaction of Ruthenium Red, the pectin of apples in store is mainly concentrated in the middle lamella, the substance of which may easily be continuous through the pits in the walls, hence forming the irregular rings of stainable material. The distinction between the crescents, discs and bands of Carré and Horne does not appear to be a valid one, because they are all part of the middle lamella which is being looked at from a different angle in the three cases. The conclusions based on such distinctions by these authors cannot be maintained until we know far more about the nature and structure of the cell-wall than we do at present.

Several determinations of the amounts of cuticle in stored apples were made, as it was thought that since external changes go on at the skin surface under storage conditions (e.g., sweating at the skin), this might indicate that further depositions were being made on to the cuticle. Most of the determinations, however, showed that the amount of cuticle present was within the range of those of the mature apple before going into storage, but some showed an increase in amount. Markley and Sando (9), however, found a definite increase in the amount of cuticle substances during storage.

The amount of cuticle deposited in apples grown under a deficit of potassium was considerably less than that of those grown with a normal potassium supply. The same methods were used as in the previous cases mentioned and the figures obtained were 288 in apples grown with a normal potassium supply and 224 for apples which were starved of potassium. In the latter case, too, the cuticle tended to be very irregular.

(*b*) *The " Eye " of the Apple.*

The structure of the " Eye " or calyx end of the apple is of importance in view of the occurrence of the disease known as " Eye-Rot," which occurs under orchard as well as under storage conditions.

" Eye-Rot " caused by *Nectria galligena* has been recorded on Worcester Pearmain apples while still on the tree by Dillon-Weston (4). The rot always begins at the calyx end and spreads laterally. The diseased fruits ripen and colour prematurely. The top of the apples becomes slightly flattened and this increases during the progress of the disease. When this disease develops under storage conditions, the apples probably go into storage with spores or hyphæ present in the calyx region. There are two possible places in which they may occur. The spores may be present on the surface of the calyx cup, in which position they may germinate and gain an entrance into the tissues of the apex of the apple under favourable conditions. Infection may, however, be present in the form of hyphæ which are unable to gain an entrance until some change in the cell-walls lining the calyx cavity has taken place. Finally hyphæ may be present in the moribund parts of the sepals and from here they may grow down into the tissues at the apex of the apple.

The calyx cup is lined by a layer of cork of very varying thickness owing to the extensive cracking which takes place on its outer surface. The cracks in Bramley's Seedling are sometimes so deep that only one or two layers of suberized cells separate the cavity from the ring of thick-walled cells surrounding the base of the cavity. This layer of cork continues below the stamens, cutting them off from the apex of the apple, but it dies away at the base of the sepals and merges gradually into the epidermis covering the upper surface of the sepals. In the majority of cases, therefore, the living cells at the base of the sepals are continuous with those at the apex of the apple. A layer of cork is present across the style at a short distance above the place where it becomes free from the calyx cup. The tube formed by the style which runs down into the core of the apple is lined with hairs which project from the surface layer of cells. In Bramley's Seedling, this tube may be open all the way down into the core, but usually it is blocked in places owing to the interlocking and growing together of some of the surface cells ; these become cemented together by a layer of suberin, which stains heavily with Sudan III.

In very young apples, before they have set, the calyx cavity is shallow and open since the sepals project horizontally from the apex of the apple. When the apple has set, the rapid growth of the fruit brings the base of the sepals into a horizontal position, thus closing the calyx cavity to a very large extent ; it now forms a much more favourable place for the germination of spores deposited at an earlier stage.

A large number of sections—hand sections as well as microtomed sections—were made of the " eye " of Bramley's Seedling and other varieties of apples.

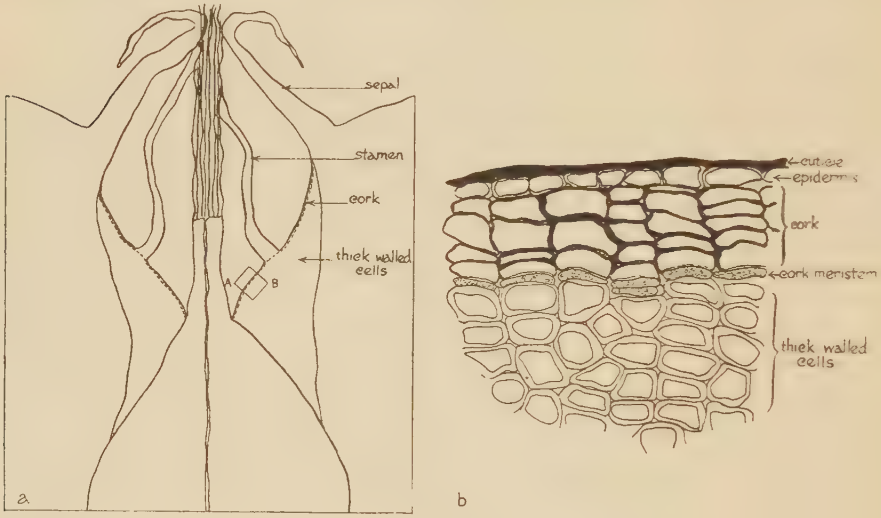

Fig. 8.

a.—Longitudinal section through apex of mature apple.
b.—Details of region A. B. before breaking of epidermis (x350).

These were stained with Cotton Blue in lactophenol so as to bring out clearly the presence of fungal hyphæ. In all the sections examined, fungal hyphæ were found amongst the cracks in the cork on the surface of the calyx cup. No signs of hyphæ were found actually in the moribund parts of the sepals, but they were often present on the inner surface of the basal part of the sepals. Up to the present time, however, no very early stages of " Eye-Rot " have been available for examination, so it is impossible to be certain how the fungus gains an entrance into the tissues of the apple. From the results of the present observations, the most likely method of attack seems to be from the deeper cracks in the cork at the base of which only a few layers of suberized cells are present. It seems quite possible that changes may go on in these walls, and in the cells below, under certain conditions, which would allow the fungus to make an entrance. However, entrance by way of the bases of the sepals is by no means precluded until apples showing the earliest stages in the disease have been examined.

SUMMARY.

1. An account is given of the morphological and cytological development of the apple Bramley's Seedling, during the season 1930.

2. The rate of increase in weight, cell size and cuticle thickness is given and correlated, as far as possible, with the weather records for the season.

3. Details are given of the varieties of epidermal structure found in the Bramley's Seedling apple.

4. Details are given of the lenticel structure of the varieties Bramley's Seedling and Ecklinville to illustrate two types of lenticel structure found in apples. The " deep " type of lenticel structure was found to be more favourable to the growth of fungal hyphæ than the " shallow " type.

5. The structure of the " eye " of the apple is described and the method of attack used by fungi causing " Eye-rot " of apples is suggested.

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THE BLACKBERRY MITES.

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Six species of Gall Mites have been recorded upon plants belonging to the genus *Rubus*, and four of these species occur upon plants of economic value, such as the cultivated Blackberry, Raspberry, Loganberry and the Himalaya Berry. These mites, namely, *E. gracilis* Nal. ; *E. gibbosus* Nal. ; *E. rubicolens* Can. ; *E. silvicola* Can. ; *E. rubierineus* Nal. ; and *E. essigi* Hassan, have not been studied to any extent in this country, and our knowledge concerning them is very limited. This does not necessarily mean that the mites are uncommon species, and it is believed that three of them are widely distributed in this country.

E. gracilis Nal. is present in most plantations where raspberries are grown, while *E. gibbosus* Nal. occurs on wild blackberries in many of our hedge rows. More recently a new species has been added to our list. It is *E. essigi* Hassan. This species was described by Hassan in 1928 (1). It occurs in Berkeley, California, and it is considered to be the cause of the blackberry disease known by the popular name of " Redberry Disease."

Eventually it may be proved that several of these mites are of economic importance in this country. It has been suggested already that *E. gracilis* Nal. may be correlated with diseases associated with the Himalaya Berry and the Raspberry (2, 3, 4). It is significant that this mite is frequently present on these plants which are growing abnormally and producing abnormal foliage and shoots. This point is further emphasized by the fact that there is some evidence to show that the well known " reversion " disease (5) of black currants is associated with the Big-Bud Mite. It will be remembered that the Big-Bud Mite is a species of *Eriophyes*, closely allied to the Blackberry Mites.

Of these mites, it has been established that one species (*E. essigi*) is of definite economic importance where cultivated blackberries are grown in the United States and to a lesser extent in this country.

It has been shown by Essig (6) in America that the Blackberry Mite (*E. essigi* Hassan) is the cause of " Redberry Disease." The same species of mite causes similar damage to cultivated blackberries in this country.

E. essigi has been recorded in this country from the Canterbury district, East Malling, Maidstone, and Kirdford, W. Sussex. It is very common in the former district and is of considerable economic importance where blackberries are cultivated in that area.

HABITS OF THE MITE.

The white, transparent mites, which are very small, being only .005 of an inch in length, hibernate during the months, from September until the end of February, under the bud scales on the shoots, and occasionally in the dried up fruits which remain on the plants all the winter. The mite population during the winter months is small, and mites can only be found by careful searching of their winter quarters. As soon as growth begins to develop in the spring, the mites become active, and they migrate to the undersurfaces of little leaflets. They feed and form colonies in amongst the fine hairs on the leaves and petioles. They continue to increase in numbers, and when the flowers begin to form, the majority of mites move from the leaves and concentrate on the flowers. The mites do not cause any apparent damage during the flowering period, but the injury caused by the mites becomes evident about two weeks before the berries are ready for picking.

EFFECT ON THE FRUITS.

The mites feed upon the droupelets as soon as the flower petals fall. They feed for the main part around the bases of the droupelets, and not near the apex of the fruits. The result is that the fruits become distorted in many instances, uneven ripening is a typical symptom, and an abnormal red condition of the fruit is another definite symptom of mite injury. This type of injury has been described in America as " Redberry Disease." This expression describes the injury so well that it is hoped this term will be used in this country for the popular name of the disease.

The berries ripen prematurely when the attack is very severe, and many of them remain on the plants in a dried up condition until the following spring.

The attack may be slight for two or three years, and then, when the season is suitable for the development of the mite, a serious outbreak may occur and spoil a large percentage of the fruits. This was noticed in a plantation at Langley, near Maidstone, recently.

The symptoms of the disease appear to be the same on the fruits of the Loganberry, Himalaya Berry and the wild and cultivated Blackberry.

When the attack is only moderate no loss is suffered by the grower, because the infested fruits are picked and mixed with the clean samples. When the attack is severe, however, the fruits are unsaleable on account of their unripened, red appearance, and their distorted shapes.

CONTROL MEASURES.

Definite control measures of a scientific nature have not been carried out in this country, but the pest became so bad in one plantation some four years ago that field control measures were carried out with the object of averting the spread of the mite to other plantations.

The blackberry bushes were sprayed with lime-sulphur (1-99) during June, just before the flowers opened. This one application of lime-sulphur did not entirely eradicate the mite, but the fruits on the treated bushes were considerably better than those on the untreated rows. No injury was observed on the bushes.

It seems reasonable to suggest that this mite will be comparatively simple to control now the life-history is better known. A strong application of lime-sulphur applied in the spring when the mites are migrating (during the latter part of February) should be effective in controlling this mite. If this treatment is found to be effective in this country, it may be possible to apply lime-sulphur, not only to control the mite, but also the Cane Spot Disease (7) of cultivated blackberries which is so common at the present time.

According to Essig (6), the early lime-sulphur treatment is cheap and effective in the United States.

The mite, *E. essigi* Hassan, is fully described below, together with details of other species which occur on plants of the genus Rubus.

ERIOPHYES ESSIGI HASSAN.

1928. Publ. Ento Univ. California. V. 4, N. 11, p. 378.

Colour transparent. Body cylindrical. Thoracic shield triangular, tapering somewhat toward caudal end ; shield ornamented with three longitudinal ridges at centre, and with less conspicuous markings at lateral margins ; shield extending over the head. There is one pair of thoracic bristles, situated near posterior margin of shield, not widely separated, and directed toward caudal end ; thoracic bristles 20μ long, rigid, conspicuous. Warts of thoracic bristles large.

Rostrum small, delicate. Legs short, fifth segment club-shaped and somewhat longer than fourth ; claws of first pair of legs 5μ long ; those of second pair 8μ long. Feathered claw small, four-rayed. Sternal ridge simple. Three pairs of coxal bristles conspicuous ; third pair 24μ long, in line with inner coxal angle.

Abdomen slender, slightly tapering toward caudal end, with approximately 70 finely punctate rings. The rings of the anal segments of dorsal surface smooth. Lateral bristles inserted well below the epigynium, very fine. First pair of ventral bristles 34μ long ; second pair 30μ long ; third pair 12μ long. Anal flap large. Caudal bristles not robust. Accessory bristles absent. Epigynium basin-shaped, large, 15μ broad. Coverflap smooth. Genital bristles directed towards anal flap, thin, slender, delicate, 20μ long.

♀ 165μ long, 40μ broad ; ♂ 110μ long, 30μ broad.

Host Plants. Wild and cultivated Blackberries. Loganberry and Himalaya Berry (Massee) ; Himalaya Blackberry, Loganberry, Raspberry and Blackberry (Hassan).

Distribution. Berkeley, California (Hassan) ; Canterbury District, East Malling, Maidstone and Kirdford, West Sussex (Massee).

E. RUBICOLENS CAN.

Canestrini, Prosp. Acarof. 1892. V. 5, p. 682.

Gall. Produces a hairy protrusion on the under-surfaces of the leaves.

Host Plants. Blackberry (Canestrini), Raspberry and Dewberry (Bagnall).

Distribution. Italy (Canestrini) ; England (Bagnall).

E. GIBBOSUS NAL.

Nalepa. Denk. Ak. Wien. 1895. V. 62, p. 634.

Gall. This species produces hairy patches on the leaves and petioles.

Host Plants. This species have been recorded upon 28 different species of Rubus, including the cultivated Blackberries.

Distribution. Europe and the British Isles.

E. RUBI WHITEHEAD.

Whitehead. Board of Agric. London (1892) 1893.

Gall. Produced small leaf galls. (Probably the same species as *E. gracilis* Nal.)

Host Plant. Raspberry (Whitehead).

Distribution. England (Whitehead).

E. SILVICOLA CAN.

Canestrini, Prosp. Acarof. 1894. V. 6, p. 780.

Gall. Produces small, ovoid, leaf galls. These galls have globular projections on their inner walls.

Host Plants. *Rubus saxatilis* L. *R arcticus* L. (Canestrini).

Distribution. Norway, Finland, Switzerland, Russia, Italy. Recorded once in England by Bagnall.

E. GRACILIS NAL.

Nalepa. N. Acta. Ac. Leop. 1891. V. 55, p. 385.

Gall. A free living species. Also produces pale hairless spots on the leaves, and causes a construction and malformation of the veins.

Host Plants. Raspberry and Dewberry (Bagnall) ; Himalaya Berry, Blackberry and Raspberry (Massee).

Distribution. Europe, British Isles, common wherever raspberries are grown.

E. RUBIERINEUS NAL.

Nalepa. Marcellia. 1914. V. 13, p. 70.

Gall. Produces rose red galls on leaves ; the galls turn brown when mature.

Host Plants. *Rubus rosaefolius* (Smith).

Distribution. Java. (Nalepa.)

SUMMARY.

The species of Gall Mites which have been recorded on plants of the genus *Rubus* are briefly described.

One species (*E. essigi* Hassan) which has been described in America as being the cause of " Redberry Disease " of blackberries is recorded in this country for the first time. The damage caused by this mite is described, together with notes on its life-cycle, distribution, etc.

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THE LAYOUT AND CONDUCT OF TWO MANURIAL TRIALS OF RASPBERRIES: TOGETHER WITH THE DEDUCTIONS WHICH CAN VALIDLY BE DRAWN.

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THE manuring of raspberries is a subject concerning which there exists little experimental evidence. Although most text-books recommend heavy dressings of organic manures, the evidence afforded by the few elementary trials, in which artificials are included, is not very illuminating.

In 1921 Chandler (1) reported that the variety of raspberry, Cuthbert, grew more vigorously when given dressings of nitrate of soda but that there was not a corresponding increase in yield ; a smaller yield per unit of cane was therefore the result.

In 1924 Grubb (4) gave a note on an experiment at East Malling, where four varieties had been compared on land receiving dressings of dung and shoddy supplemented by spring dressings of fish or meat meal, and on land from which all except green manure had been withheld. While one variety, Pyne's Royal, showed a marked increase in growth and cropping on the manured land, two had done somewhat better on the unmanured plot, and the fourth showed no difference. It is probable, however, that these results were complicated by disease.

In 1925 Wallace (9) described the growth and foliage characteristics of Pyne's Royal raspberry grown in pots and subjected to various manurial deficiencies for two seasons. The growth showed a marked falling off where nitrogen, potassium and calcium were withheld.

THE PROJECT AT EAST MALLING.

In 1927 the piece of land known as Newgates field was purchased, and of this some four acres were already planted up with Pyne's Royal raspberry canes and a further three and a half acres with the variety Lloyd George, then two years old.

These two varieties, which were known to be true to name and free from rogues, presented an admirable contrast for the purpose of manurial trials.

Pyne's Royal, a berry of generally fine quality, had yielded well previously at East Malling (5), but had proved a poor cane maker on this soil, a loam of variable depth derived from river terrace material over the Hythe Beds. These consist of alternate layers of a crystalline limestone and an amorphous calcareous sand or sandstone, known locally as ragstone and hassock respectively.

The variety Lloyd George, on the other hand, is an exceptionally prolific cane maker, yielding heavy crops of large berries.

It was therefore determined to lay down two manurial trials on these two blocks of raspberries. Perhaps the most important part of the problem was the question as to how to set about these manurial trials. Here was a large area of one crop—so much so that little in the way of detailed records could be attempted ; how far was it possible, using large plots and relying for information upon such simple observations as crop and cane growth, to answer fundamental questions concerning the manurial requirements of the plants ?

Secondary to this problem of technique the manurial issues in question appeared to be distinct. In the case of the Pyne's Royal, the production of sufficient cane seemed to be of primary importance, while in Lloyd George, in direct contrast, it appeared that the first need was to maintain cropping *without* encouraging a too exuberant cane growth.

THE MANURIAL APPLICATIONS.

(*a*) *Pyne's Royal.*

At the time of planning this experiment it was believed that the principal manurial necessity for Pyne's Royal was nitrogen and that, indeed, unless this was given, it would not be possible to grow this variety on this land at all.

Accordingly a basal dressing of 1½ cwt. per acre of sulphate of ammonia was given to all plots. This was compared with a double dressing of the same fertilizer.

These dressings were further supplemented by a more bulky nitrogenous manure in the form of shoddy. The shoddy used was the wastage from carpet factories ; and besides being of an exceptionally high grade manurially (16 per cent. ammonia), was in a very fine state of division. Comparisons were made between single and double dressings of this manure at the rates of 10 and 20 cwt. per acre in the presence and absence of a double dressing of sulphate of ammonia. Thus the nitrogen dressings range from the single dressing of sulphate of ammonia giving approximately 35 lbs. of nitrogen per acre, to a double dressing of sulphate of ammonia and a double dressing of shoddy, giving about 360 lbs. of nitrogen per acre.

Finally, the possible importance of potash applications was considered, and plots which received 3 cwt. per acre of sulphate of potash were compared with plots from which potassium was withheld, in combination with the varying quantities of nitrogen. There were, therefore, twelve different sets of plots in the experiment:—

1. 1½ cwt. of sulphate of ammonia.
2. 3 cwt. ,, ,,
3. 1½ cwt. of sulphate of ammonia + 3 cwt. sulphate of potash.
4. 3 cwt. ,, ,, + ,, ,, ,,
5. 10 cwt. shoddy + 1½ cwt. sulphate of ammonia.
6. ,, ,, + 3 cwt. ,, ,,
7. ,, ,, + 1½ cwt. ,, ,, + 3 cwt. sulphate of potash.
8. ,, ,, + 3 cwt. ,, ,, + ,, ,,
9. 20 cwt. ,, + 1½ cwt. sulphate of ammonia.
10. ,, ,, + 3 cwt. ,, ,,
11. ,, ,, + 1½ cwt. ,, ,, + 3 cwt. sulphate of potash.
12. ,, ,, + 3 cwt. ,, ,, + ,, ,,

(*b*) *Lloyd George.*

In this case the problem was mainly to maintain crop, and nitrogen dressings appeared likely to be of secondary importance, if indeed they were not detrimental in producing too much cane at the expense of the crop. Accordingly no basal dressing was given, the control plots receiving no manure. These were compared with others receiving a light dressing of sulphate of ammonia at 1½ cwt. per acre.

In this experiment the potash problem was examined in more detail.

Previously at this Station and elsewhere both sulphate of potash and kainit, but in particular the latter, had been suspected of causing damage when applied late to raspberry canes. Therefore, while plots receiving potash were compared with those from which it was withheld, in the presence and absence of nitrogen, the relative merits of these two forms of potash were examined by applications of sulphate of potash and kainit. The quantities of each were adjusted to give the same amount of potash.

The kainit used in this case contained no magnesium, the quantity applied being 11½ cwt. per acre to correspond with a dressing of 3 cwt. of sulphate of potash.

Further, in this experiment the question of time of application of the potash dressing was considered, and accordingly, after the first season when they were all applied in March, half the potash applications were put on in December and the other half in March.

The sulphate of ammonia has been applied throughout in March.

The treatments in this trial were thus ten in number and were as follows:—

1. Nil.
2. 11½ cwt. kainit, applied in December.
3. ,, ,, ,, March.
4. 3 cwt. sulphate of potash, applied in December.
5. ,, ,, ,, ,, March.
6. 1½ cwt. sulphate of ammonia.
7. ,, ,, ,, + 11½ cwt. kainit applied in December.
8. ,, ,, ,, + ,, ,, ,, March.
9. ,, ,, ,, + 3 cwt. sulphate of potash applied in December.
10. ,, ,, ,, + ,, ,, ,, March.

THE LAYOUT OF THE EXPERIMENTS.

These experiments were among the first trials laid down at this Station, in which the methods developed by Fisher at Rothamsted were used (2, 3, and 7). They are therefore valuable in themselves, quite apart from the information obtainable from them with regard to the manuring of raspberries, being a test of the adaptability of these methods of field experimentation, to horticultural crops. So much for the point of view of the statistician, who has to lay out trials of sufficient accuracy to answer the questions put to him. From the point of view of the pomologist, they give an answer to the question as to how far large scale field trials, unsupplemented by pot experiments or detailed laboratory observations of any kind, are of any value to him in elucidating his manurial problems.

Size of Plot.

Previous information regarding the best size of plot for raspberry experiments was very meagre, almost the only critical data concerning the variation in this crop being that supplied by Grubb (5). As a result of his early experience in harvesting two varieties at East Malling in 30 ft. lengths of row, he adopted as his plot in a further variety trial, a single length of row 56 ft. long and repeated this plot four times.

The results of this trial recently published (6) show that after four years cropping, only comparatively large differences (30 per cent.) in the total crop in four years were significant ; and that the cropping in individual years was even more variable, as is usually the case owing to seasonal effects. It was also apparent in this trial that the incidence of disease was likely to have an influence on the variation in cane growth and crop of many varieties. Therefore, before the size of plot and number of replications was decided upon, in order to test the variability of the *material* a survey of the cane growth of both varieties was made in the autumn of 1927. This was done by measuring the cane growth of measured lengths of row selected at random throughout the field.

From this survey the following information was obtained:

In the Pyne's Royal area there was a very marked variation in the number of canes to a given length of row and many stools missing. Further, the cane lengths of different stools varied greatly. This variation was not in any way connected with position, but was distributed more or less at haphazard over the field.

On the other hand, in the Lloyd George piece there was apparently a very distinct positional variation, the length of cane increasing markedly from the north-west corner of the plot, where the cane was poor, to the south-east, where growth was rampant. Local variations were, however, much less than in the case of Pyne's Royal.

As a result of these observations on the variability of the material the following sizes of plots were agreed upon.

(*a*) *Pyne's Royal.* Here large plots were decided upon in order to try to even up the local variations ; since with small plots, whereas one plot might have a full plant, another might have practically no cane at all. Each plot therefore consisted of three lengths of row, each twenty yards in length. The rows running north and south, the plots were separated from each other from east to west by a single buffer row, and by eighteen feet of unmanured row within the rows from north to south, to prevent carrying over of manures in cultivation. The manures were applied all over the ground up to each guard row (see Fig. 1). The rows being somewhat less than six feet apart, the manured area of each plot worked out at .032 of an acre, the actual cropping area being .025 acre. Each plot was repeated eight times.

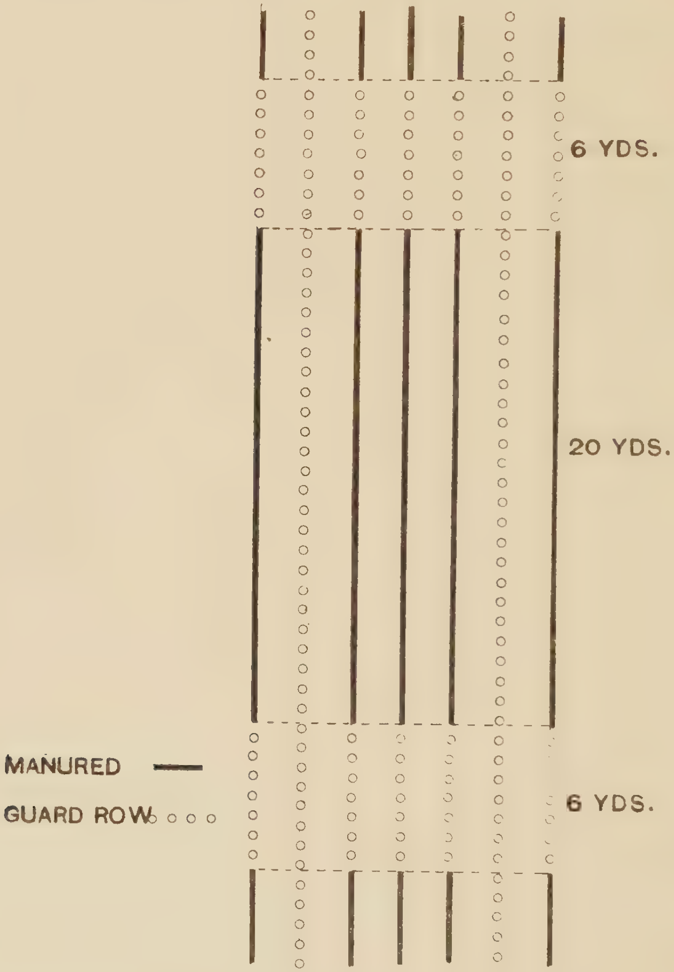
(*b*) *Lloyd George.* The plots in this case were rather smaller, since the local variations appeared to be less important, and to fit them into the available ground the shape had to be modified. Thus each plot consisted of two lengths of row twenty-five yards in length surrounded by the same guards as in the former case, giving a manured area of .029 of an acre or a cropping area of .019 acre. Again each plot was repeated eight times.

METHOD OF ARRANGEMENT.

The method of arrangement selected for this trial was that of randomized blocks (2, 3, and 7)—a method peculiarly suited to accurate manurial trials, where a large number of treatments are to be compared. Each field was therefore divided into eight blocks and one plot devoted to each treatment was contained in each block. These blocks were so arranged as to sample possible variations in yield due to position in different parts of the field. Thus in the Pyne's Royal there were twelve separate treatments, making ninety-six plots in all, covering an area of four acres, about one and a half acres of which was occupied by the guard rows.

FIG. I.

Diagram showing the size and description of each plot in the Pyne's Royal experiment.

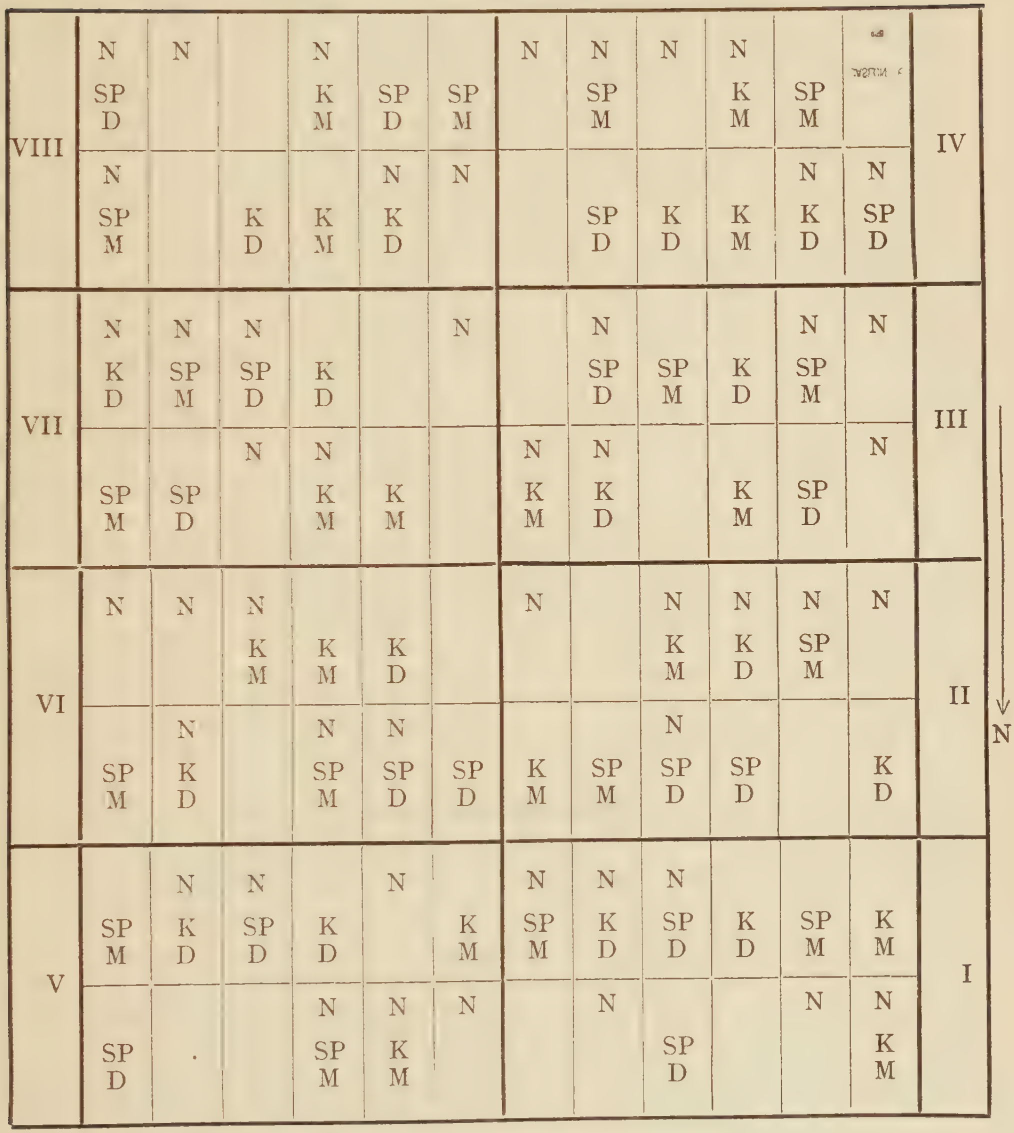


In the case of Lloyd George, the primary issue was the reaction of nitrogen and potash and thus it was necessary to have an equal number of plots receiving

FIG. 2.

Manurial Trial of Lloyd George Raspberries.

Method of Arrangement—Eight Randomized Blocks.



VIII	N SP D	N		N K M	SP D	SP M	N	N SP M	N	N K M	SP M		IV
	N SP M		K D	K M	N K D	N		SP D	K D	K M	N K D	N SP D	
VII	N K D	N SP M	N SP D	K D		N		N SP D	SP M	K D	N SP M	N	III
	SP M	SP D	N	N K M	K M		N K M	N K D		K M	SP D	N	
VI	N	N	N K M	K M	K D		N		N K M	N K D	N SP M	N	II
	SP M	N K D		N SP M	N SP D	SP D	K M	SP M	N SP D	SP D		K D	
V	SP M	N K D	N SP D	K D	N	K M	N SP M	N K D	N SP D	K D	SP M	K M	I
	SP D			N SP M	N K M	N		N	SP D		N	N K M	

KEY. N = 1½ cwt. Sulphate of Ammonia per acre.
SP = 3 cwt. Sulphate of Potash per acre.
K = 11½ cwt. Kainit per acre.
D = Potash applied in December.
M = Potash applied in March.

the treatments O, N, K, NK, S.P. and N.SP. In order that the potash might be applied at two different times, however, and at the same time the minimum of eight plots per individual treatment maintained, there had to be sixteen plots of each of these primary treatments. In the case of the potash plots, eight received their applications in December and eight in March. There were thus again eight blocks, in each of which were twelve plots, but in this case there were only ten individual treatments, the nil and nitrogen plots being duplicated in each block. There are thus again ninety-six plots altogether and the total area required was about three and a half acres, about one and a half acres of which again was occupied by buffers. A plan of the Lloyd George trials is shown in Fig. 2.

Before the first manurial applications were made soil samples were taken from each of the ninety-six plots in both trials at two levels, nine inches and eighteen inches below the surface. These samples were air dried and stored. At the time of taking there appeared to be distinct differences in soil texture and depth on different plots. Recent examination by auger borings to five feet has shown that while in some parts of the field there is over three feet of a mellow, light loam, in others the soil below twenty-one inches is harsh and unweathered, consisting of either a very compact and yet gritty material or a loose, coarse sand.

The first dressings were put on in March, 1928 ; and in this year only, the potash dressings on all plots were applied in this month.

(i) *Crop Records.*

The crops were harvested each year and weighed in tenths of a pound at each picking. The number of pickings varied in different years according to the length of season, but, roughly speaking, each plot was picked over about twice a week ; where it was not possible to pick the whole ninety-six plots in one day, care was taken to pick a definite number of blocks, so that the same number of plots undergoing each treatment were picked on the same day.

The size of the plots and total acreage under each experiment made these trials essentially commercial. The plots were cultivated and trained as far as possible under commercial conditions and the same conditions applied to the harvesting of the crop, except in so far as the pickers were paid by the day. For this reason harvesting ended with normal commercial picking. The amount of fruit left unharvested was in no case large, but in the case of Lloyd George a certain quantity of autumn fruits were not included in the crop.

The picking of the crop in these large scale trials demanded very careful organization, since at the height of the season as many as 100 pickers with no special experience in experimental work were employed. The first essential was therefore the careful demarcation of plots and a proper supervision of the pickers. It is interesting to note that what at first seemed a very formidable task has been easily accomplished. Although at harvest time only one technically trained recorder was employed on the plots, and then only to weigh and record the fruit picked, the errors in picking have been unimportant.

(ii) *Cane Growth.*

That a preliminary survey was made of the cane growth over the whole field has already been noted, but no individual plot measurements were taken until after the first year's crop had been harvested. In the winter, 1928-29, cane measurements were made on half the plots (four blocks) in each experiment, one row being measured only in each plot. The records taken were, number of stools, number of canes, length of cane per stool and total length of cane. This has been continued annually on the Lloyd George plot ; but on the Pyne's Royal plot it was quickly realized that, owing to the local variations in numbers and length of cane in individual stools, this record was of little value. Accordingly, in the last three seasons the total length of cane has been measured for all plots. All measurements were taken in decimetres and telephoned direct to the laboratory, where they were recorded on a Burroughs adding machine.

(iii) *Other Pomological Records.*

In October, 1931, Mr. T. Wallace inspected the Lloyd George experiments and pointed out the prevalence of typical raspberry leaf scorch upon certain plots. Accordingly counts of scorched canes were made on random samples of fifty canes in each plot.

GENERAL HISTORY OF THE EXPERIMENTS.

(i) *Weather conditions during the period as affecting the crop.*

The effect of the moisture conditions on cropping and cane growth is somewhat complicated by the positional differences in the field, especially in the case of Lloyd George. It is therefore necessary to divide this experiment into two halves, since the effect of weather conditions has not been the same on the East and West sides of the field.

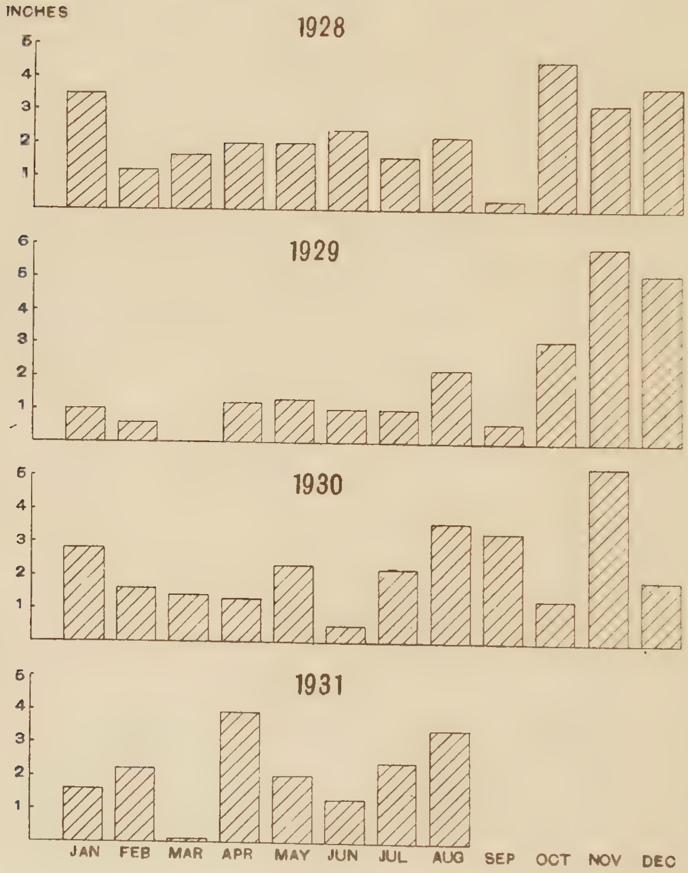
Fig. 3 illustrates the monthly rainfall experienced at East Malling during the period of the trial to the date of writing. Table I shows the average crop in cwts. per acre for all plots, irrespective of treatment, but not including buffers, in each year, and the average cane growth in metres per plot (fifty yards in Lloyd George and sixty yards for Pyne's Royal).

1928. The summer of 1928 was rather a wet one for East Malling conditions. Picking commenced on July 2nd and continued until the 30th. The crop was comparatively good for Lloyd George, but very poor for

Pyne's Royal. The dry September probably influenced the cane growth, which was very poor and variable in Royal and rather low for Lloyd George. Although, as will be seen, variations in crop of the latter due to soil differences were very high, this was not shown in the cane growth, the difference between the east and west sides of the plot being much less well marked than in other seasons (see Fig. 5).

FIG. 3.

Rainfall at East Malling, 1928-31.



1929. After a wet autumn in 1928, the rainfall in the first seven months of 1929 was exceptionally low. The season, however, was late and picking started on July 18th and continued until August 9th. The crop of the Pyne's Royal was about the same as the year before, but the cane growth very meagre. The *crop* of Lloyd George was lower than in the previous year, especially in the eastern portion of the field. This was probably due to unusually low growth of the fruiting cane formed in this part of the field in 1928. The mean *cane growth* in this year was somewhat higher than in 1928, but this was due to a big rise on the east side, the west side being rather lower than the previous year.

1930. In this year a wet winter was followed by a moderately rainy spring. June was, however, a dry month, but the rainfall in July was above the average. Picking started on July 2nd and continued until the 29th. The crop and cane growth were on the whole better in both experiments. This was again confined to the east side of the Lloyd George trial, that of the west side remaining about the same as 1929.

TABLE I.

Average Cropping of Pyne's Royal and Lloyd George Raspberries in cwts. per acre—seasons 1928-1931, *and Cane length per plot in metres.*

	Royal.		Lloyd George.	
	Crop.	Length of Cane.	Crop.	Length of Cane.†
1928	7.9	—	52.2	590
1929	8.2	141	46.2	618
1930	11.4	218	52.5	668
1931	13.6	209*	65.6	659

* Measured August 1931 before grubbing.

† Mean of 48 plots.

1931. A mild, though not very wet, winter and a very dry March were followed by a wet and sunless summer. Picking started on July 7th and continued until August 6th. The crop was the heaviest so far recorded for both varieties, the Lloyd George averaging over three tons to the acre. At the end of this season the Pyne's Royal experiment was considered to have served its purpose and the canes were grubbed in August. The cane length given therefore scarcely represents the full growth for the season.

Summing up the general history of the four year period, it will be seen that the Lloyd George have on the whole cropped well, taking into consideration the fact that one-sixth of the plots have received no manure at all. The Royals, on the other hand, have yielded poor crops throughout ; and although there has been a small progressive increase in yield during the period, in no case has a paying crop been harvested, even the heaviest yielding plots giving barely a ton an acre in 1931, the year of heaviest crop, after four years of manurial applications.

(ii) *Variation.*

Apart from differences between plots receiving different treatment, the variation in an experiment may be divided into two main parts, the variation in the material itself and that which can be ascribed to differences in position. Table II shows the coefficient of variability (standard deviation as percentage of the mean) for fruiting in each year, firstly including the variation due to position and, secondly, when that part of the variability has been eliminated.* The two experiments in this respect show a complete contrast.

TABLE II.

Coefficient of Variability for Crop of Pyne's Royal and Lloyd George Raspberries.

Year.	Pyne's Royal.		Lloyd George.	
	Total.	Eliminating position.	Total.	Eliminating position.
1928	27.2	21.1	30.0	15.7
1929	22.7	20.0	12.8	9.9
1930	22.7	20.2	11.4	8.9
1931	21.6	17.8	16.9	10.3

(*a*) *Pyne's Royal.*

The Royals show a very high inherent variability. It is true that this has decreased in the last season, but compared with that in Lloyd George, it has throughout remained high. The soil variation is, however, not very marked and though, for example, one block remained near the top in crop throughout the four seasons, the other blocks have varied considerably from year to year.

This high inherent variation, on which the accuracy and thus the success of the experiment must depend, has made it difficult to deduce any adequate results therefrom, despite the magnitude of the project. The plots started with different potentialities and it is only by observing the cropping and growth in relation to that in previous years that, until the last season, any results have been seen at all.

The reasons for this variation were probably in the main connected with disease, apart from the fact that Pyne's Royal is undoubtedly a " poor doer " on this soil and that none of the treatments applied radically altered its character. The major portion of the stools are now known to have been infected with

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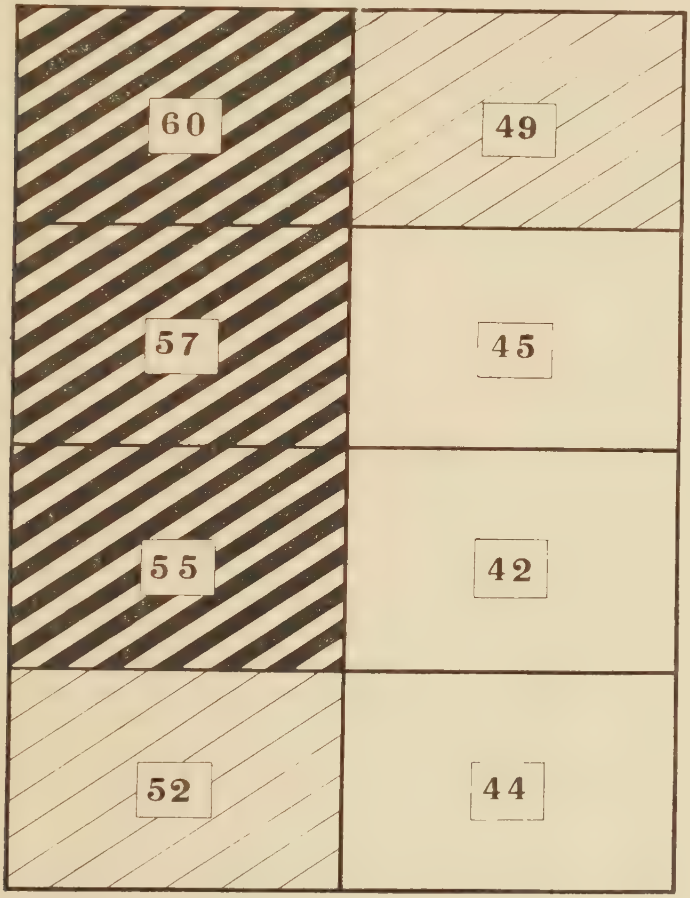
* This first figure is obtained by calculating the amount of variation in each set of plots treated alike, the coefficient of variability being based on the mean of these amounts. An estimate of the amount of variation due to position can be obtained from the yields of the eight blocks, since each block is alike in that it contains one plot devoted to each treatment. The second figure is obtained by eliminating this amount of variation from the total previously found.

Mosaic disease ; and although it is not suggested that the whole mortality of canes and complete stools can be ascribed to this cause, there is little doubt that it was a most important factor in the excessive variation in the vigour of growth of the stools. The other disease which was very prevalent on this plot was Cane Blight (*Leptosphæria coniothyrium*) and this was probably responsible for much of the damage.

FIG. 4.

Influence of position on crop of Lloyd George in different parts of the field, as illustrated by total yield in cwts. for four years.



(Cropping area of each block = ¼ acre approx.)

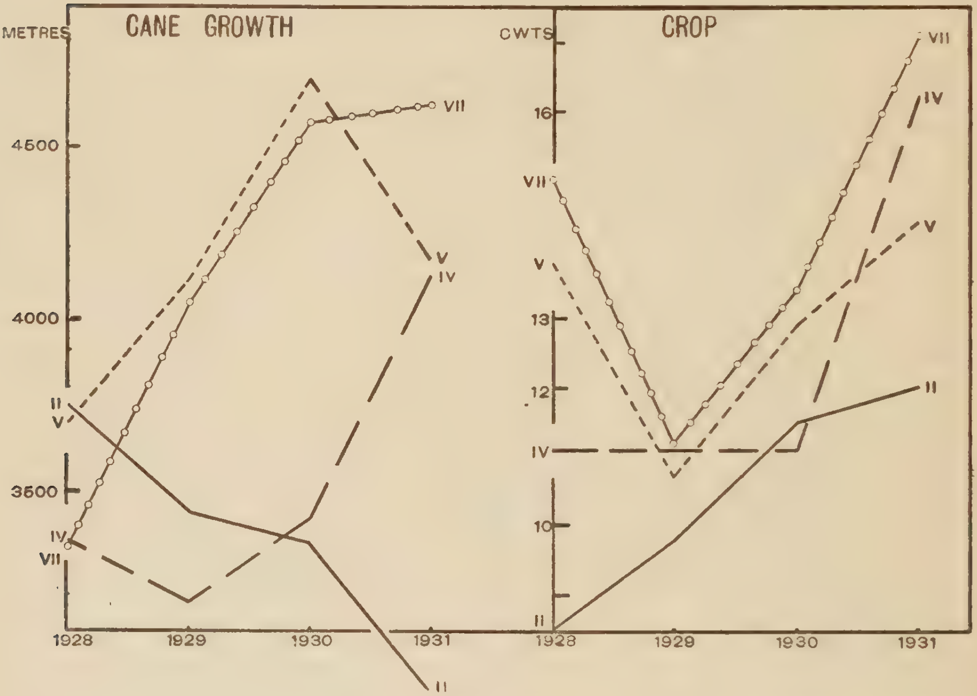
While on the whole the data collected from this trial have been disappointing, the results to be shown prove that the trial has been by no means useless. The experience here also drives home the precept that, not only is true material an essential in all experiments, but that varieties should be chosen which are suitable to the soil and which can be kept reasonably free from disease.

(*b*) *Lloyd George.*

The variation in the Lloyd George trial is of a very different nature. Here there is a definite influence which can be ascribed to position and Fig. 4 shows that block 8 in the extreme south-east corner of the field has in four years yielded nearly a ton more fruit on a cropping area of about a quarter of an acre than has block 2. Not only has there been a difference in crop, however, but, as has already been noted above, Fig. 5 shows the response to seasonal influence has not been the same throughout the field. These differences have occurred every year, but it is noteworthy that those in crop have been greater in years of high summer rainfall, and that differences in cane growth seem to correspond more nearly to the rainfall in August and September (see Fig. 3). It is interesting to compare this result with those of Hoffman and Schlubatis, who

Fig. 5.

Influence of seasonal conditions irrespective of treatment on growth and cropping of Lloyd George in four blocks in different parts of the field.



found that both growth and fruiting were correlated with the height of the water table (8).

A further explanation connecting these seasonal influences on variation with the availability of potash is suggested by the results to be shown.

When this source of variation is eliminated, it will be seen that the inherent variation is small in comparison with that in the Royal's (Table II), so much so that the experiment has turned out to be one of the most accurate, so far carried out at East Malling, it having been possible to be certain of differences of less than ten per cent.

This trial is in many respects a complete justification of the experimental method used. Such soil differences as have been shown here are by no means unique, and the fact that not only have there been these enormous differences in cane growth and cropping associated with different parts of a three and a half acre field, but that the behaviour from year to year of the variety in this respect has also differed, demonstrates clearly the value of an unbiassed distribution of the differently treated plots and a method which can estimate and eliminate these differences.

MANURIAL RESULTS.

(*a*) *Pyne's Royal.*

Table III gives the average yields, number of canes, and total length of cane for each of the twelve different treatments in the Pyne's Royal trial during the four year period. Each of these averages is that of eight plots and the general mean for all plots and the appropriate standard errors will be found at the bottom of the table. The method used in examining the data was the "analysis of variance." Descriptions of the method have been given by its author and others in various publications (2, 3 and 7). In the present instance it will suffice to say that, in the analysis, the various component parts of the total amount of variation can be separated and compared. Thus the portion of the variation which is contributed by differences due to treatment is compared with that due to the errors of the experiment and the significance of the difference tested. In this method a standard deviation which is common to the whole experiment is calculated, and from this the standard error appropriate to the means of different sets of plots derived.

(i) *Cane Growth.*

The average cane growth per plot during the last three years is illustrated in Fig. 6. The first sign of a response to manurial treatment was found when the growth in 1930 was considered in relation to that in 1929. As the diagram shows, at this time all plots which received shoddy were increasing in cane

TABLE III.

Average Crop and Cane Growth of Pyne's Royal Raspberry, 1928-31.

		1928.	1929.			1930.			1931.			
		Crop. lbs.	Crop. lbs.	No. of canes.	Total length of cane. cm.	Crop. lbs.	No. of canes.	Total length of cane. cm.	Crop. lbs.	No. of canes.	Total length of cane. cm.	Total Crop.
No Shoddy	1N*	24.5	24.7	272	157	32.3	348	196	35.3	326	181	116.8
	2N	20.4	21.4	261	151	32.2	378	207	38.0	374	209	112.0
	1NK	21.5	24.5	249	147	32.9	370	214	39.9	343	190	118.8
	2NK	20.8	23.4	249	144	33.5	376	215	40.1	342	192	117.8
Single Shoddy	1N	23.1	24.7	224	131	32.0	345	204	36.5	338	194	116.3
	2N	22.4	20.9	221	119	28.0	344	195	31.3	342	192	102.6
	1NK	24.3	24.3	260	152	32.6	371	228	41.7	392	241	122.9
	2NK	21.9	23.3	234	138	31.3	396	234	38.9	359	210	115.4
Double Shoddy	1N	21.6	22.4	226	136	30.2	386	222	35.5	368	211	109.7
	2N	21.6	23.8	245	143	33.3	369	226	36.3	395	223	115.0
	1NK	20.1	21.5	238	144	32.2	390	238	42.3	412	245	116.1
	2NK	20.0	20.0	238	136	28.9	369	238	36.5	380	224	105.4
General Mean	..	21.8	22.9	243	141	31.6	370	218	37.7	364	209	114.0
S. E.†		1.6	1.6			2.3			2.4			

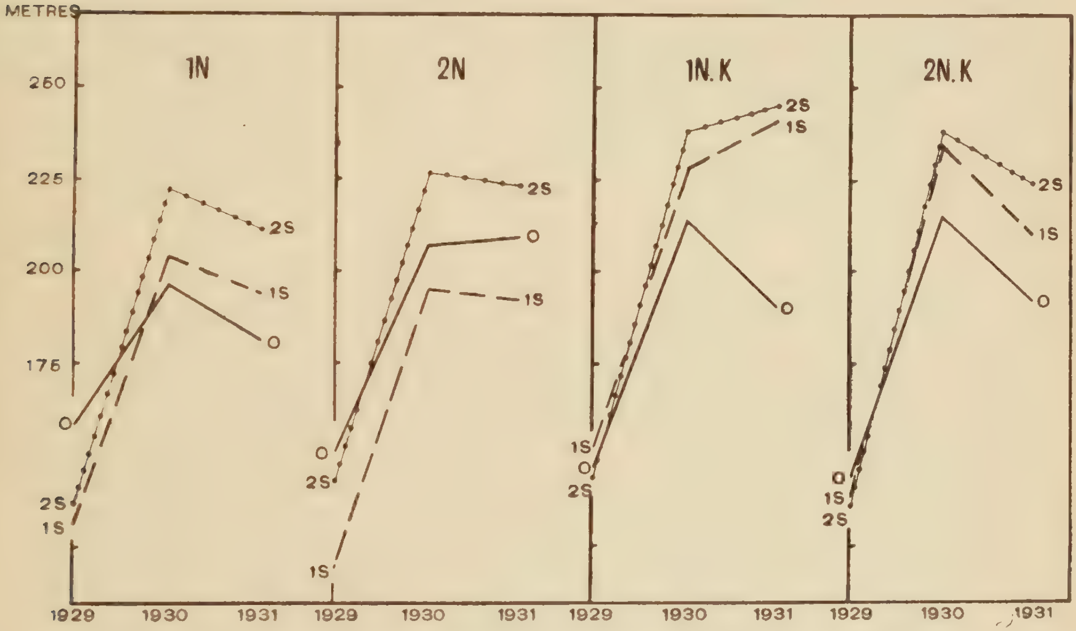
* 1N = 1½ cwt. S. of Ammonia. K = 3 cwt. S. of Potash. 2N = 3 cwt. S. of Ammonia.

† Standard Error of Mean of eight plots.

growth more rapidly than those which did not. There was, however, little difference at that time between the plots which received a single and those which received a double dressing. At the same time there was a suggestion that the plots receiving a double dressing of sulphate of ammonia were doing better than those which had only the single dressing, and also that the addition of potash was beneficial to growth.

Fig. 6.

Influence of manurial treatments on total cane length per plot of Pyne's Royal, 1929-1931.



1N =1½ cwt. Sulphate of Ammonia.
2N =3 cwt. Sulphate of Ammonia.
K =3 cwt. Sulphate of Potash.
1S =10 cwt. Shoddy.
2S =20 cwt. Shoddy.
} per acre.

In 1931 the results were more complex, but in this year significant differences in actual growth appeared. Shoddy again on the whole increased cane growth; but though a double dressing undoubtedly proved superior to no shoddy, owing to the set of plots receiving a double dressing of sulphate of ammonia and a single dressing of shoddy (1S—2N) being very low, the position of the single shoddy dressing was less certain; but it seems probable that, while the smaller dressing has in itself improved cane growth, the plots receiving it have not done as well as those getting the double dressing.

With the exception of plots receiving no shoddy, the double dressing of sulphate of ammonia showed no increase over the single dressing and, indeed, where potash was applied as well, it has been definitely depressing.

The sulphate of potash, on the other hand, has increased growth only where the single dressing of sulphate of ammonia was applied. Thus, while the mean growth of the plots receiving no potash and single sulphate of ammonia was 195 metres, where potash was applied the growth was 225 metres. On the other hand, where double sulphate of ammonia was applied, the mean growth without and with potash was 208 and 209 metres respectively.

This suggests the need for a correct balance between nitrogen and potash for cane growth. This was evidently achieved where shoddy and a single dressing of sulphate of ammonia were applied with potash, but the balance was upset where the sulphate of ammonia was increased. It is noteworthy that such a comparatively small additional dressing of readily available nitrogen should have made so much difference, whereas an extra dressing of the presumably slower acting shoddy did not have this effect.

(ii) *Crop.*

The yields of the differently manured plots during the four years are illustrated in Fig. 7. The diagram is divided into three parts and in each the four treatments 1N, 2N, 1NK and 2NK are compared firstly where no shoddy was applied, secondly where a single, and thirdly where a double dressing of shoddy was applied. The first point that is made clear from these graphs is that there is no sign that shoddy in any way directly influenced the cropping, in spite of the effect on cane growth previously shown.

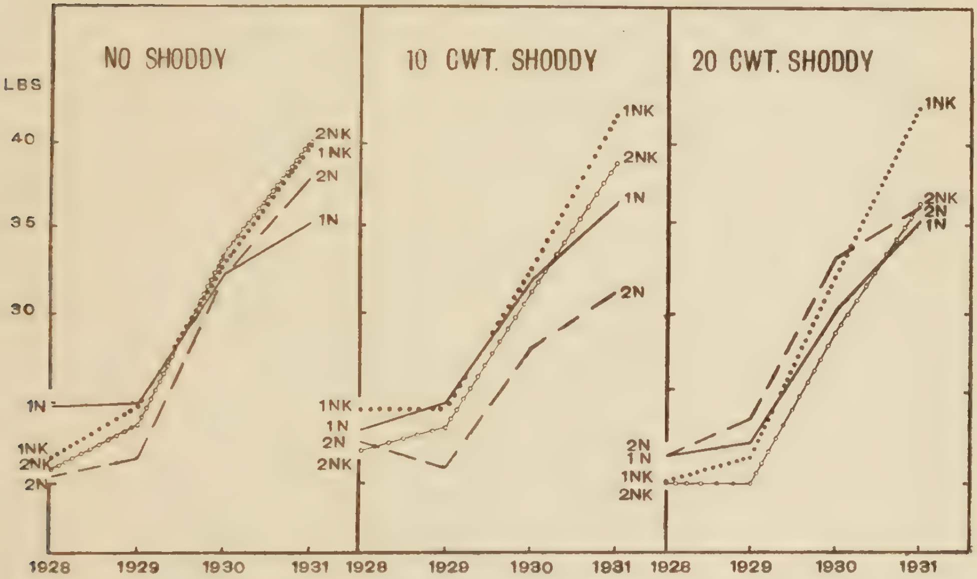
When the interaction of the two comparisons involving sulphate of ammonia and sulphate of potash is considered, it appears that, except where there was no shoddy applied, the extra dose of sulphate of ammonia was nearly always depressing, especially in the last season. Sulphate of potash was, on the other hand, invariably beneficial to cropping ; and in the last season the actual crop was significantly greater on the potash plots than on the no potash plots, the average crop for all the forty-eight plots receiving potash being 40 lbs. or 14.4 cwt. per acre, and that for those receiving no potash 35.5 lbs. or 12.8 cwt. per acre, a difference of 2.75 lbs. (1 cwt. per acre) being significant.

To sum up the results of this trial, it would appear that though shoddy increased cane growth, extra nitrogen in artificial form was detrimental not only to growth but also to crop. It was, however, to some extent beneficial where no shoddy was given. The increase in cane due to shoddy was not up to the end of the experiment accompanied by a larger crop. Potash was beneficial both to crop and directly or indirectly to growth, though the action was slow and the increases small, the largest difference in crop in 1931 being about 11½ lbs. per plot, representing a difference in yield of about 4 cwt. per acre.

It seems likely, however, when the four years' history is reviewed, that this difference would become more marked with time. This is borne out by the results so far obtained on Lloyd George—which variety will remain under observation for a further period.

FIG. 7.

Influence of manurial treatment on cropping of Pyne's Royal.



KEY.

1N = 1½ cwt. of Sulphate of Ammonia.
2N = 3 cwt. of Sulphate of Ammonia. } per acre.
K = 3 cwt. of Sulphate of Potash.

(*b*) *Lloyd George.*

Table IV shows the average crop per plot, and number of canes and cane length per half plot of the differently treated plots in the Lloyd George trial. This trial, in contrast to the former, has throughout the four seasons shown definite responses to the differences in treatment. In this connection the action of the potash and nitrogen dressings has been so far dependent upon one another, that it seems better to consider the interaction of these two issues first and the question of time of application of potash after.

TABLE IV.

Cropping and Cane Growth of Lloyd George Raspberry in four seasons.

	1928.*			1929.			1930.			1931.			
	Crop. lbs.	No. of canes per half plot.	Total length of cane per half plot.	Crop. lbs.	No. of canes.	Total length metres.	Crop. lbs.	No. of canes.	Total length metres.	Crop. lbs.	No. of canes.	Total length metres.	Total Crop.
Nil	**111**	293	289	**100**	309	290	**114**	301	297	**138**	334	295	**463**
K.D†	—	—	—	**98**	285	286	**109**	301	297	**137**	346	299	**454**
K.M	**110**	299	294	**93**	306	294	**109**	301	278	**147**	334	270	**459**
SP.D	—	—	—	**101**	328	319	**123**	330	344	**148**	383	359	**488**
SP.M	**116**	295	277	**105**	301	317	**118**	318	317	**143**	362	324	**482**
N	**112**	292	299	**101**	307	308	**111**	320	349	**129**	335	315	**453**
N.KD	—	—	—	**99**	304	316	**108**	324	349	**141**	381	373	**467**
N.KM	**119**	296	297	**97**	318	314	**114**	316	345	**152**	385	356	**482**
N.SPD	—	—	—	**100**	331	332	**116**	343	396	**156**	392	391	**484**
N.SPM	**112**	303	313	**110**	334	330	**123**	334	387	**150**	367	363	**495**
General Mean	**113**	296	295	**100**	311	309	**114**	319	334	**143**	357	330	**470**
S.E.	4·5			3·5			3·6			5·2		21·5	

* All potash applied in March this year.

† K = Kainit. SP = S. of Potash. N = S. of Ammonia. D = Potash applied in December. M = Potash applied in March.

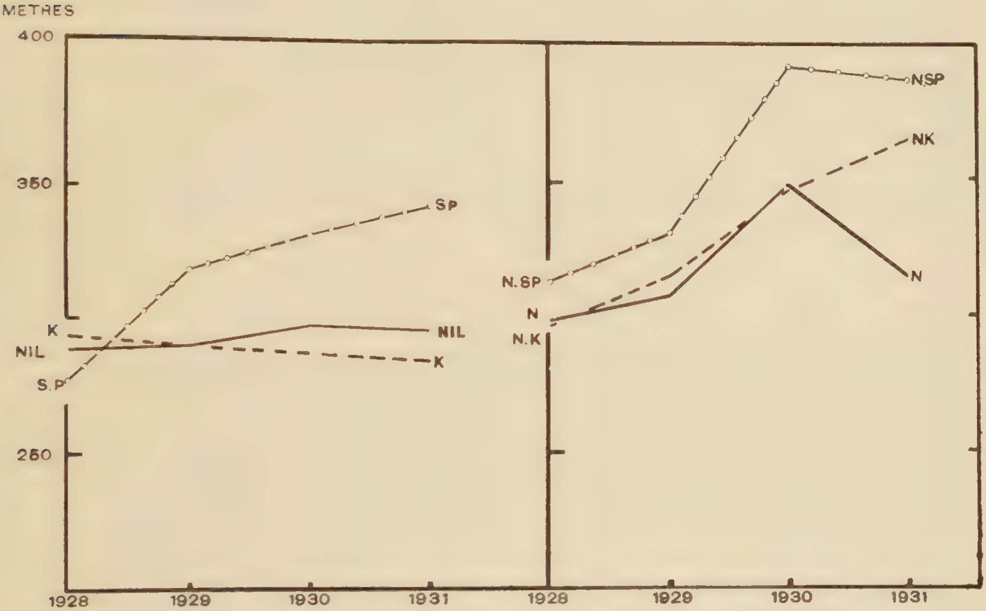
INTERACTION OF NITROGEN AND POTASH.

(i) *Cane Length.*

Up to and including the year 1930 the effect of the sulphate of ammonia dressing has been to increase the amount of cane, both as regards actual number of canes and total length (see Fig. 8). This has been the case not only where nitrogen was the only manurial constituent applied, but also in the presence of both kainit and sulphate of potash.

FIG. 8.

Influence of manurial treatment on cane growth of Lloyd George irrespective of time of application.



Nil =No manure.
N =1½ cwt. Sulphate of Ammonia.
SP =3 cwt. Sulphate of Potash. } per acre.
K =11½ cwt. Kainit.

Kainit has not in these first three seasons proved beneficial in producing cane ; where applied alone the result has not differed from the no manure plots, and where applied with sulphate of ammonia it has produced no additional increase in cane. Sulphate of potash has, on the other hand, given considerably more cane, both in the presence and absence of nitrogen, since 1928, when by itself it had no effect. As will be seen, this may be due to the fact that it was applied in March.

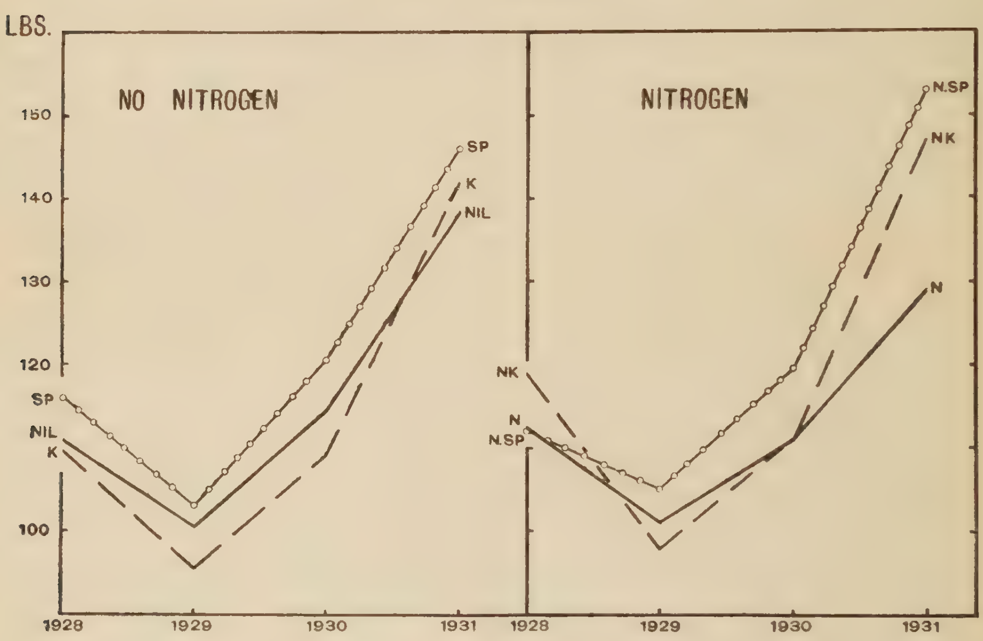
In 1931 the effect of the sulphate of ammonia, where applied alone, was greatly reduced ; but in the presence of potash it was still beneficial. Kainit in this year produced more cane in the presence of nitrogen, but not alone.

These results apply irrespective of position in the field. It is interesting to note that, with the dressings applied, the expected superfluity of cane has not materialized, though whether this will be the case on the N.SP plots in future seasons remains to be seen.

(ii) *Crop.*

Fig. 9.

Influence of manurial treatment on crop of Lloyd George raspberry irrespective of time of application.

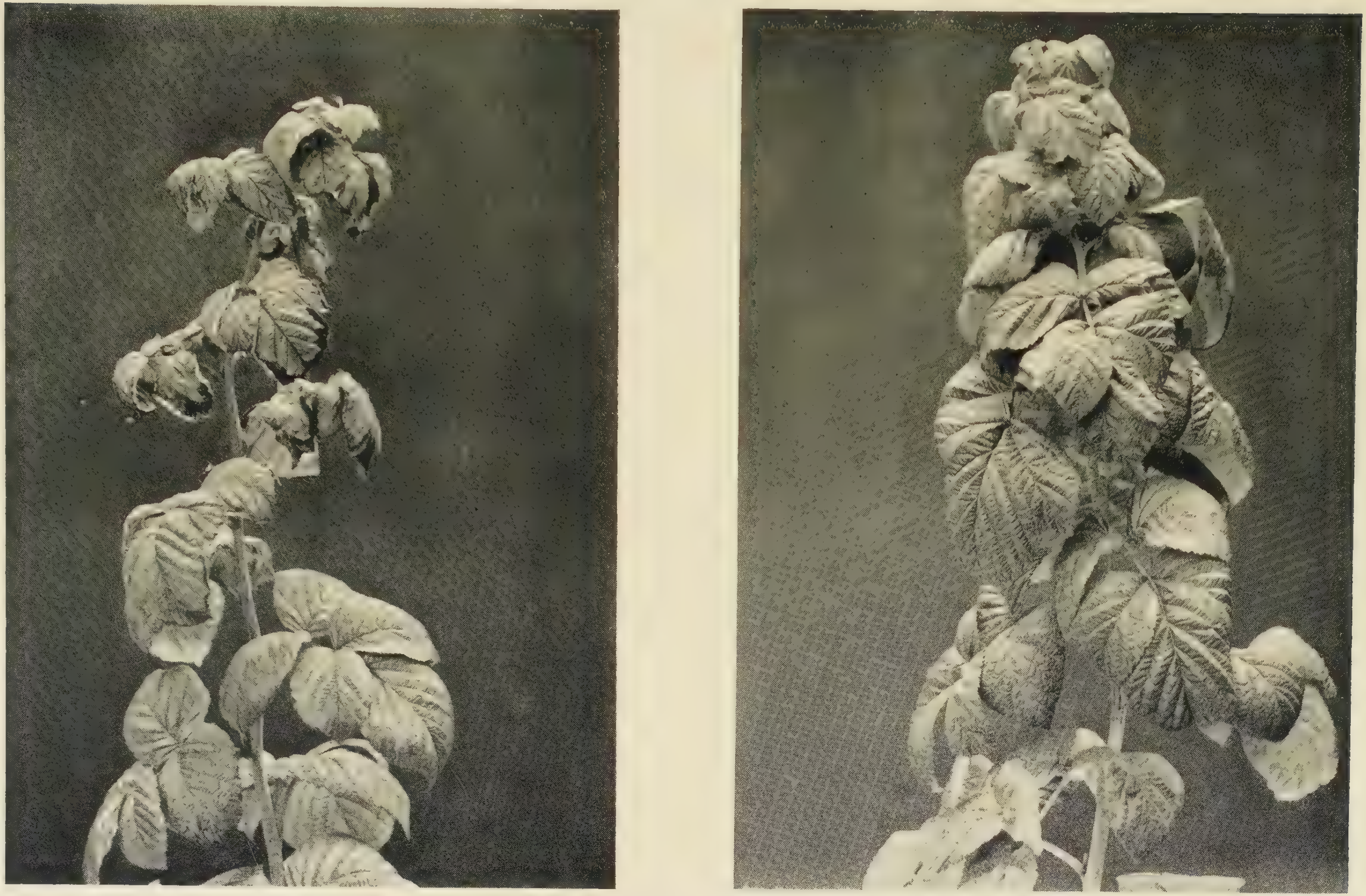


Nil =No manure.
N =1½ cwt. Sulphate of Ammonia.
SP =3 cwt. Sulphate of Potash. } per acre.
K =11½ cwt. Kainit.

In spite of the additional amount of cane where sulphate of ammonia was applied, there has never been, up to the date of writing, any increase in crop which can be attributed to this fertilizer, *where applied alone* (Fig. 9). Indeed, in the last two years it has had a distinctly depressing effect, thus in 1931 nitrogen alone produced 129 lbs. per plot (or 59 cwt. per acre), whereas the control plots averaged 138 lbs. (63.5 cwt. per acre). In the last season (1931),

FIG. 10.

Comparison of Healthy and Scorched canes in Lloyd George raspberry. September, 1931.



Cane showing Leafscorch.

Normal Cane.

however, *in the presence of potash*, whether kainit or sulphate, the nitrogen has produced a larger crop: that for the N.-S.P. plots being 153 lbs. per plot against 145 lbs. for S.P. alone—a difference of 3.7 cwt. per acre—and that for the N.K. plots being 146 lbs. against 142 lbs. per plot for K. alone—a difference of 2 cwt. per acre.

With regard to kainit, the sum effect of both times of application seems to have depended rather upon the season. Thus, in 1928, kainit by itself had no effect, but in the presence of nitrogen seems to have done some good. In 1929 and 1930 the effect of the kainit was depressing, whether given with sulphate of ammonia or alone. However, in 1931, in spite of having made comparatively poor cane growth in the previous season, the crop was considerably better, both in the presence and absence of nitrogen, than the respective controls.

Sulphate of potash, on the other hand, has in the last three seasons proved beneficial to cropping; and the plots receiving this fertilizer have always had the most crop as well as the most cane. Though the presence of nitrogen made little difference until 1931, in this year the effect of the sulphate of potash was substantially increased by its addition.

The sum effect of these results is to confirm the conclusion arrived at from the Pyne's Royal experiment; that is the necessity for a proper balance between nitrogen and potash, the more vital necessity on this soil appearing to be potash.

(iii) *Leaf Scorch.*

If further evidence of the need for potash be required, the condition of the field at the end of the period shows it very clearly. In September, 1931, symptoms of potash deficiency, as described by Wallace in his pot experiments (9), began to appear on the plots from which potash had been withheld. These consisted in the earlier stages of a reddish-brown coloration of the veins and margins of the leaves. As the symptoms became worse, the brown colour changed to the typical leaf scorch associated with potash deficiency in the leaves of other fruits. The scorch appeared first at the edges of the leaves, increasing until the whole leaf was shrivelled up (see Fig. 10).

These symptoms were so well marked that a record was taken. Fifty canes were selected at random from each plot and these divided into the following categories according to the condition of the leaves:

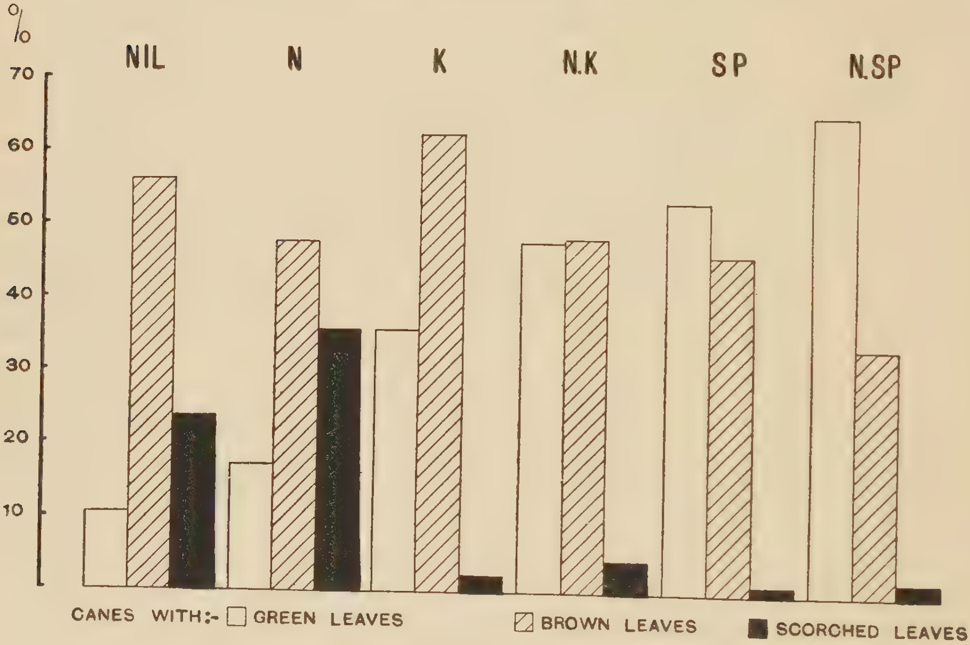
1. healthy green leaves,
2. leaves with brown or red markings,
3. scorched leaves.

Fig. 11 shows the result of this survey, the three columns allotted to each treatment representing the percentage of canes falling in to each category.

It is seen at once that the scorched leaves were most frequent where nitrogen was put on alone, there being some 36 per cent. falling in to category 3. The control or no manure plots were the next worst, having about 23 per cent. scorched. Where potash was put on the percentage in this category was negligible, the sulphate plots being somewhat better than the kainit. The proportion of canes with green healthy leaves was much the highest on the sulphate of potash plots and very low on the nil and nitrogen only plots.

FIG. 11.

Influence of manuring on percentage of canes showing leaf scorch in Lloyd George, September, 1931.



Nil =No manure.
N =1½ cwt. Sulphate of Ammonia.
SP =3 cwt. Sulphate of Potash.
K =11½ cwt. Kainit.

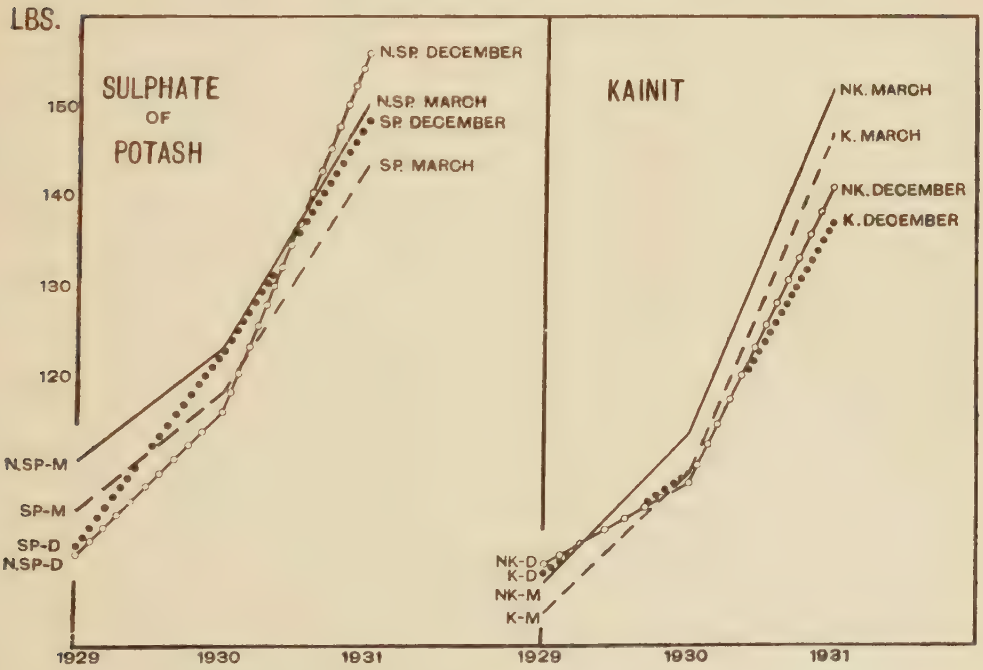
The percentage of canes with brown leaves is a little more difficult to understand, since the kainit plots have an exceptionally high proportion in this category. The high proportion of canes with brown leaves may be simply due to the partial action of the kainit as compared with sulphate of potash. On the other hand, it seems possible that a proportion of these are not due to potash deficiency at all, but are in some way connected with damage caused by the kainit. It is, however, quite impossible to differentiate between the two sets of symptoms by eye alone. It is worthy of note that where nitrogen was put on with potash—though the number of canes in category 3 was somewhat higher—the amount of browning was greatly reduced. These symptoms are easy to see on the whole field, but the intensity of scorch was much greater on the west side of the plot than upon the east side. Indeed, although the response to treatment is relatively the same all over the field, whereas the demonstration on the west side could hardly be missed, on the east side it is not nearly so striking. This suggests that the positional variation as shown in cane and crop is intimately connected with the available potash in different parts of the field.

Time of Application of Potash.

The question of time of application of potash seems to be dependent upon season and upon the kind of potash applied. The effect upon crop is shown in Fig. 12.

Fig. 12.

Influence of time of application of potassic manures on crop of Lloyd George raspberry in three seasons.



N =1½ cwt. Sulphate of Ammonia.
SP =3 cwt. Sulphate of Potash.
K =11½ cwt. Kainit.
D =Potash applied in December.
M =Potash applied in March.

In 1929, 1930 and 1931 sulphate of potash had a better effect upon cane growth when applied in December. The effect on crop, however, has been more complicated ; for there was a distinctly better effect from the March application in 1929, little or no difference in 1930, and in 1931 a distinct advantage from applying the sulphate of potash in December. There has been no clear influence of time of application of the kainit on cane growth, but the effect on crop has been the direct opposite of that in the case of the sulphate. Thus in the dry season of 1929 the kainit was less detrimental when applied in December ; in 1930 there was no distinguishable effect, and in the wet summer of 1931 the kainit applied in March was distinctly better than where applied in December.

The explanation of the effect of kainit may lie in the weather conditions immediately following its application. Thus in one case the detrimental substances may be washed out at once, in another they may remain in the ground long enough to be absorbed by the plant. It is difficult to see why the sulphate has acted in this way.

CONCLUSION.

In conclusion, it may be suggested that the results, up to the present date, of these two experiments amply justify the time and labour expended upon them. With regard to planning of future experiments, two points emerge very clearly. In the first place uniform material free from disease, especially Mosaic, is essential. Secondly, a proper distribution of plots on the ground in such a way that each different treatment has an equal chance of experiencing any soil differences which may appear, can need little further justification when the enormous soil differences in the Lloyd George trial are taken into account.

Given better material, it should be possible to reduce the size of such experiments by using smaller plots ; but guard rows, which are essential, must always materially increase the size of all manurial trials. It should be remembered, however, that the smaller the plot the more accurate must be the methods of recording. The number of such plots must, of course, depend upon the number of treatments and the differences which it is hoped to show.

The most important manurial information, which has been obtained from these experiments with very different varieties, is the necessity on this soil for a proper balance between nitrogen and potash. Applications of nitrogen, up to a point, may produce more cane, but if this is overdone, the cane growth may be reduced. For crop, however, nitrogen alone has been not only ineffective but inadvisable except in the presence of potash, which, where applied as the sulphate, has always proved beneficial in the quantities applied.

Of minor results, it appears that kainit is, on the whole, less suitable than sulphate of potash, for though in a single season it may prove beneficial, in others it may do harm. The question of time of application for potash needs further evidence over a number of years before any definite recommendation can be made.

Finally, the writer finds himself in somewhat of a quandary in tendering his acknowledgments ; since he can only claim to be the mouthpiece of that considerable section of the staff at East Malling, who have at one time or another been connected with these experiments. He is therefore compelled to mention only those who have actually assisted in the work of recording in the field. Of these, Mr. W. S. Rogers and the late Mr. W. Appleyard carried out the recording in 1928 and Mr. W. A. Bane in 1929 ; and more recently practically the whole work of recording has been done by Miss B. Carter and Miss A. B. Beakbane. The responsibility for the careful organization during the picking season and the marketing of the crop has been throughout in the hands of Mr. A. C. Painter.

SUMMARY.

1. Two large scale manurial trials on Pyne's Royal and Lloyd George raspberry have now been carried out for four seasons at East Malling.

2. These experiments, which were the first trials at this Station in which the method of randomized blocks was used, are in themselves ample justification of the method.

3. In connection with the planning of further trials these two trials demonstrate two points very clearly: (*a*) Even in large scale trials with comparatively large plots, uniformity of material and freedom from disease are essential. (*b*) Differences due to position on the ground may prove much greater than differences due to the results of manurial treatment ; so that a proper distribution of replicated plots is absolutely necessary.

4. The results indicate that, on this soil, *with two varieties of very different habit*, a proper balance between nitrogen and potash is the secret of successful manuring of raspberries. Nitrogen may, up to a point, produce more cane by itself, but has never increased the crop except in the presence of potash. Indeed, alone it has been depressing in both cases.

5. Sulphate of potash, which has always been beneficial, is, on the whole, superior to kainit.

6. Time of application for potash depends upon kind and season.

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